BIOPHYSICAL ASPECTS OF PLANT TISSUE CULTURES

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INTRODUCTION

The effects of radiation on the physiological and metabolic processes of living organisms are of prime interest to many investigators in biophysics. The processes of growth and respiration are vital properties of any living tissue and should provide exceptionally good indices of overall radiation damage to the living system. On the basis of the numerous effects of X-rays and other ionising radiations on living systems which have been noted, one should expect that the overall metabolism of the system, measured by the quantity and rate of gaseous exchange, would be rapidly and notably affected by the radiation. Even a brief survey of the work dealing with this aspect of radiation damage, however, shows that such an expectation has not been corroborated by experiment. But, the effects of radiation on growth and development of living organisms confirm expectation. Morphological changes in embryos as a result of gene mutation and chromosome aberration induced by radiation have been observed and studied for some time. Less obvious than these gross effects are the microscopic changes, attributed to ionising radiation, that take place in the immediate neighborhood of a cell. These changes probably influence cellular metabolic processes; hence, they affect the subsequent development of the cell and the organism as a whole even though a gene mutation or chromosome change is not involved.

A survey of the literature also reveals that through expediency most investigators concerned with radiation effects on metabolic processes have worked with animals high up the evolutionary scale and that comparatively little work has been done with simple organisms. The fundamental metabolic processes are the same in all forms of life. But, the higher forms of life contain many different cell types with certain specialized metabolic processes. Moreover, when such an organism is irradiated, the activities of the different cells taking part in a metabolic function may be speeded up, slowed down, or even stopped. Consequently, the degrees to which these cells participate in certain reactions may also change. It is not surprising, then, that radiation effects on respiration have escaped detection and that the interpretation of growth anomalies, other than those caused by direct action, has not met with complete success.

The purpose of this investigation is to study the growth and respiration of a living organism of a different nature, which may be grown under controlled environmental conditions, and to determine whether or not X-rays influence the normal respiratory process and growth of the organism.

Corn root tips are therefore used because it is known that they may be excised and grown in sterile nutrient solution. In comparison with many animal tissues they contain relatively fewer different types of cells.

To attempt to reduce an organism to its constituent cells and to study these cells as elementary organisms is a project of fundamental

importance in the solution of basic biophysical problems. The technique of segregation of cells, tissues, and organs from the body as a whole and their maintenance as isolated units under the most optimum and fully controlled conditions possible is a difficult task. But, by reducing the complexity produced by many different types of cells functioning simultaneously, the technique of isolating simple cells or cell structures can provide the means of breaching many basic problems. Cognizant of this basic standpoint, plant physiologists for many years have proceeded methodically to grow cells and small groups of cells in nutrient solutions in order to study various phenomena associated with their growth. However, they are still faced with many difficulties, which have been only partially overcome, in the cultivation of plant tissues. The chief difficulties are: (1) the selection of the proper organic and inorganic substances and their concentrations for optimal growth and development of the tissue which in its natural state is not immersed in a free nutrient solution, and (2) the excision of a plant part which exposes delicate, vital parts and gives rise to shock to the excised portion in its removal from the whole body. In spite of these and other difficulties which resulted in many futile experiments, considerable successful work has been carried out by different workers since about 1930. Although excised plant cells have not been grown in artificial nutrient media with complete success, there is no doubt that normal growth of the cultures will eventually be realized.

The body of this research report is divided into six main sections. Chapter I is concerned with the growth of excised corn roots

in artificial nutrient media and the effect on primary growth of the growth hormone, indoleacetic acid. In Chapter II a related growth study, the lateral root formation as a function of indoleacetic acid, is investigated. The use of the quantitative statistical method yields considerable information about this auxiliary growth phenomenon. In Chapter III the respiration of excised tissues as affected by different concentrations of the growth hormone indoleacetic acid is studied. The problem of determining whether or not the respiration of an excised root is altered when its primary growth is retarded or enhanced is discussed. The tissue cultures were exposed to doses of X-radiation varying from 100 to 1400 roentgens and the oxygen uptakes of the irradiated cultures were measured. Experimental results are presented and discussed in Chapter IV. The primary growth and lateral root formation of the cultures which were irradiated are briefly taken up in Chapter V. Some previous investigations have made use of the bioelectric potentials of organisms as indices of radiation damage. Without going into the mechanisms or origins of bioelectric potentials, consideration has been given to the measurement of the bioelectric potentials of the plant tissue cultures to align the present work with past growth studies and to lend support to the usefulness of the bioelectric potential as a measure of radiation damage. The bioelectric potential measurements are discussed in Chapter VI.

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CHAPTER I

THE GROWTH OF EXCISED ROOTS AS A FUNCTION OF INDOLEACETIC ACID CONCENTRATION

Introduction

The growth of a plant takes place in certain localised regions where cell division occurs. These regions are called meristematic regions. At the tips of roots, primary growth is initiated by an apical meristem. The region of cell extension lies just behind the meristematic region of the root. This region is mainly responsible for root elongation. Behind the region of elongation lies the region of cell differentiation. The growing end of a root is contained in the apical 1 to 2 mm length of the root (1, 2). Therefore, an excised root tip of 1.5 cm length contains a comparatively large proportion of meristematic and cell elongation regions and is an excellent culture for growth studies.

The meristematic regions of cell division are seats of intense assimilation of carbohydrates and proteins. To use these and other foods for the maintenance of the process of cell division, the cells require numerous growth regulating substances. Many of these substances are enzymes or coenzymes while others may be classed as hormones or vitamins. All take part in essential reactions of the metabolic mechanism.

Plant growth hormones play a specific role in the regulation of the growth of one part of a plant as compared to another. In general, they are synthesised in one tissue and then travel to another site for action. They are organic substances and, like enzymes, are extremely active in small concentrations.

The most important plant growth hormone is indoleacetic acid (IAA). This hormone and other related compounds are called auxins. The auxins

are prominent in the control of stem and root growth, lateral bud inhibition, fruit growth, and many other physiological activities of the plant. Small quantities of auxin are synthesized in apical root meristems (3). Unrestricted growth may be controlled by a specific enzyme also synthesized by the plant cells. This enzyme may break down indoleacetic acid to an inactive substance (4).

The metabolic role of the naturally occurring auxin indoleacetic acid is not completely known. There is evidence that the auxins act in a catalytic or regulatory capacity in some phase of the carbohydrate or organic acid metabolism of plants (5, 6). Because of the very low concentrations necessary to produce such striking growth effects, it is possible that auxin may operate as the prosthetic group of an enzyme.

When a root is excised from the parent plant it is deprived of a part of its normal source of auxin, since most of the auxin is synthesized in the leaves and transported to the roots. Normal cell elongation is therefore inhibited since the proper quantity of auxin is necessary for optimal growth of the cells. An artificial source of auxin must become available to the excised root. Because of inherent differences between plants, the optimum auxin concentration for normal growth varies greatly with different tissues and is not known for all plants. Relatively higher than optimum auxin concentrations usually exert an inhibitory effect on this phase of growth. The optimum concentration of indoleacetic acid for growth of roots of oat seedlings is about 1.6×10^{-8} grams per liter and concentrations above about 10^{-7} grams per

liter inhibit growth of the roots (7). Bonner and Koepfli, in their study of the growth of avena roots, found that concentrations of indoleacetic acid higher than 10^{-4} mg per liter inhibited growth (8, 9). The determination of the concentration of indoleacetic acid which enhances the elongation of excised corn root tips is one of the problems of this investigation.

Experimental Procedure

The culture of excised plant tissues in sterile media is the technique used in this experiment to determine: (1) whether or not the primary growth of plant tissue cultures of corn root tips is affected by indoleacetic acid, and (2) the concentrations of IAA which result in enhancement and inhibition of primary growth.

Corn seeds of a hybrid variety (Dixie No.18 Field Corn) are washed in a 1% solution of $Ca(ClO)_2$ for 20 minutes. For germination, the seeds are transferred to petri dishes under sterile conditions with sterile forceps, three seeds to each dish. The petri dishes contain two layers of blotting paper, cut to the size of the dish, moistened with distilled water and have previously been autoclaved for 20 minutes at a pressure of 17 pounds per square inch and a temperature of 255° F. The seeds are germinated for about six days in a darkened room equipped with an ultraviolet germicidal lamp for purification of the air. Then, root tips of about 1.5 cm length are excised using sterile techniques. The root tips are immediately transferred to 125 ml Erlenmeyer flasks containing 50 ml of White's nutrient solution augmented by the various

concentrations of indoleacetic acid. The flasks with their nutrient media have been autoclaved previous to the transfer of the root tips. One root tip only is grown in each flask. The flasks are stoppered with cotton-gauze plugs and capped with 50 ml beakers. They are placed in an incubator where the cultures are grown at 20° C for a total period of 15 days.

The effect on primary growth of the roots is studied for six different concentrations of indoleacetic acid in the nutrient solution. These concentrations range from 10^{-6} to 10^{-1} mg IAA per liter nutrient medium. Since there is some variability in the length of individual cultures, especially in the more advanced stages of growth, ten cultures are grown in their nutrient media for each separate concentration of auxin. The mean value of the lengths of ten cultures represents satisfactorily the primary growth of any individual root over the period of time, or any interval of the period, for which the study is made. The length of the cultures is measured with a flexible ruler placed outside the culture flask. Measurements of the length of each root culture are made at the time of transfer and then at daily intervals for the first seven days. After this period, the lengths are measured at odd days up to and including the fifteenth day.

Results

Figure 1 shows the mean lengths of the root cultures in cm over the 15 day period for the various concentrations of indoleacetic acid. C_0 is the control, the subscript zero indicating that the cultures are

grown in nutrient media containing no auxin; C_1 indicates the growth curve of cultures grown in nutrient containing 10^{-1} mg auxin per liter, etc. The growth curves are noticeably different for cultures grown in nutrient media containing different concentrations of indoleacetic acid. Concentrations of 10^{-5} and 10^{-6} mg IAA per liter nutrient enhance growth of the root cultures while 10^{-1} and 10^{-2} mg per liter show an inhibitory effect. When the nutrient contains 10^{-3} mg auxin per liter, the growth curve of the root culture parallels that of the cultures grown in nutrient containing no auxin. The mean lengths of the root cultures for these two cases are the same after 15 days growth.

To determine whether or not the differences in length of the tissues which had indoleacetic acid treatment and the controls are significant, the data at the 15 day period are analyzed statistically. The Student's t-test is applied to ascertain whether or not the mean length of the root cultures grown in a nutrient having a certain concentration of auxin belongs to a different population or statistical distribution than the mean length of those roots not subjected to auxin treatment; or, in other words, to find whether the difference in the growth may be attributed to the presence of auxin in the nutrient or merely to the natural variability of growth among several specimens. If t is large, the probability that the mean lengths belong to the same distribution is small; i.e., the mean lengths are significantly different. If t is small, the corresponding probability is large and the mean lengths most probably belong to the same statistical group; i.e., the difference in the lengths is not significant. Table 1 gives the tabulated results

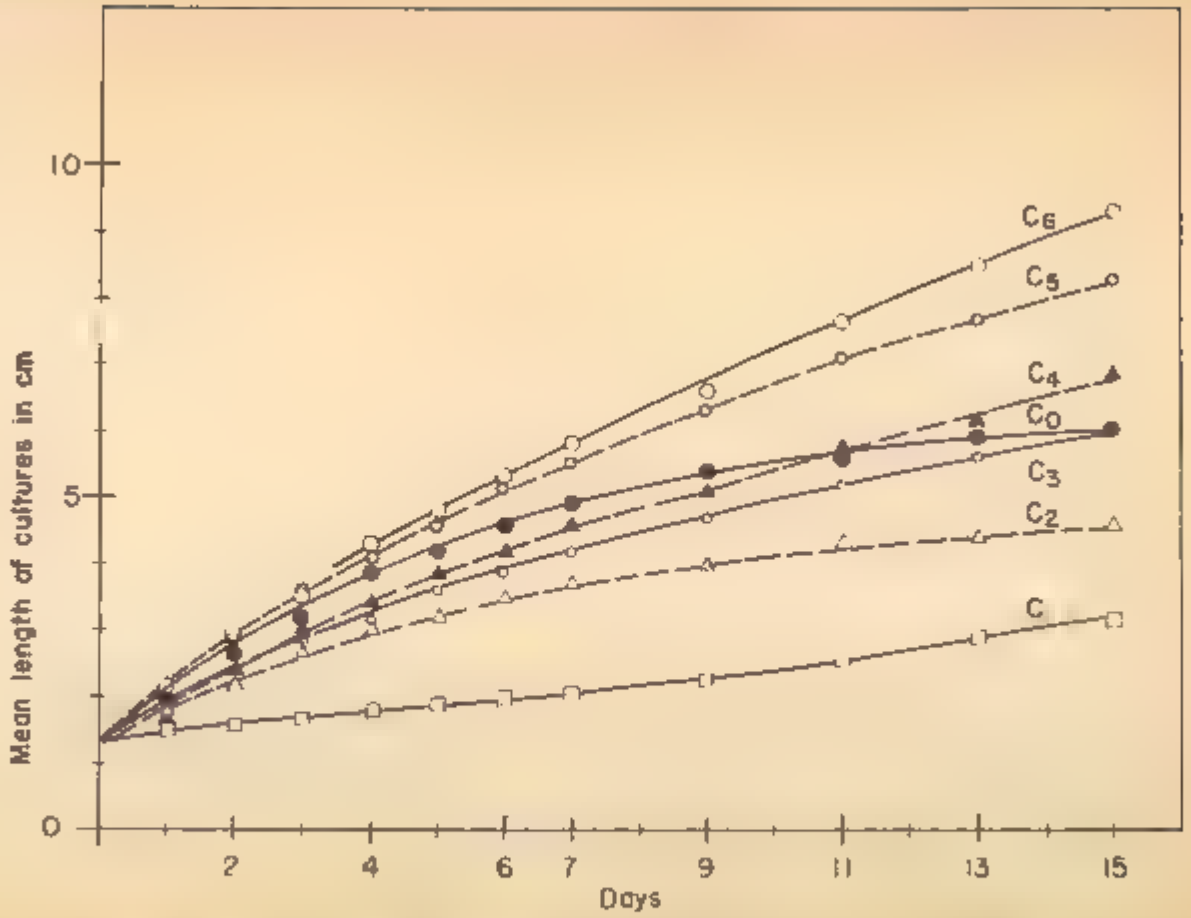

Fig 1 - Growth of tissue cultures for concentrations of IAA ranging from 0 to 10^{-1} mg per liter nutrient solution

of these analyses and shows that three cultures grown in nutrient containing 10^{-3} and 10^{-4} mg IAA per liter do not have lengths significantly different than those grown in nutrient having no auxin. However, the t-test shows that there is a significant difference between the mean lengths of the control roots and those grown in nutrient solutions augmented by 10^{-1}, 10^{-2}, 10^{-5}, and 10^{-6} mg IAA per liter, respectively. The first pair of concentrations inhibit root culture growth while the latter pair of concentrations enhance growth of the roots.

Figure 2 shows the mean culture lengths after 15 days growth expressed as percentages of the corresponding mean length of the control for the various concentrations of indoleacetic acid. The concentration of 10^{-6} mg per liter effects a mean length of one and one-half times the control while 10^{-1} mg auxin per liter causes the roots to attain only half the length of the control roots. The final mean length of the cultures increases linearly with decreasing concentration of indoleacetic acid in the nutrient medium.

After the first few days, the growth curves of Figure 1 seem to become linear. If straight lines of the form $y = a + bx$ can be fitted to the data, the slopes of these lines are the uniform growth rates which the tissue cultures attain after the initial non-linear period of growth. The data are satisfactorily fitted to straight lines by the method of least squares for the 4 to 15 day growth period (see Table 2). The growth rates decrease almost uniformly with increasing concentrations of auxin, as shown in Figure 3.

TABLE 1

MEAN LENGTHS OF TISSUE CULTURES, THEIR STANDARD DEVIATIONS, AND THE SIGNIFICANCE OF THE DIFFERENCES OF THE MEAN LENGTHS OF AUXIN TREATED CULTURES FROM THE MEAN LENGTHS OF UNTREATED CULTURES AFTER 15 DAYS GROWTH

	C_0	C_6	C_5	C_4	C_3	C_2	C_1
Mean Length, $\bar{x}$	6.1	9.3	8.2	6.9	6.1	4.6	3.3
Variance of Mean, $V_{\bar{x}}$	0.130	0.259	0.377	0.137	0.094	0.089	0.010
Standard Deviation, $\sigma_{\bar{x}}$	0.4	0.5	0.6	0.4	0.3	0.3	0.1
$(\bar{x}_1 - \bar{x}_{C_0}) = \bar{d}$	—	3.2	2.1	0.8	0.0	-1.5	-2.8
$V_{\bar{x}_1} + V_{\bar{x}_{C_0}} = V_{\bar{d}}$	—	0.389	0.507	0.267	0.224	0.213	0.141
Standard Deviation, $\sigma_{\bar{d}}$	—	0.6	0.7	0.5	0.5	0.5	0.4
t_{18}	—	5.2	3.0	1.6	0.0	3.0	7.0
Prob. $\bar{x}_1$ and $\bar{x}_{C_0}$ same distribution	—	<.001	.01-.001	.20-.10	1.00	.01-.001	<.001
Is Difference Significant?	—	Yes	Yes	No	No	Yes	Yes

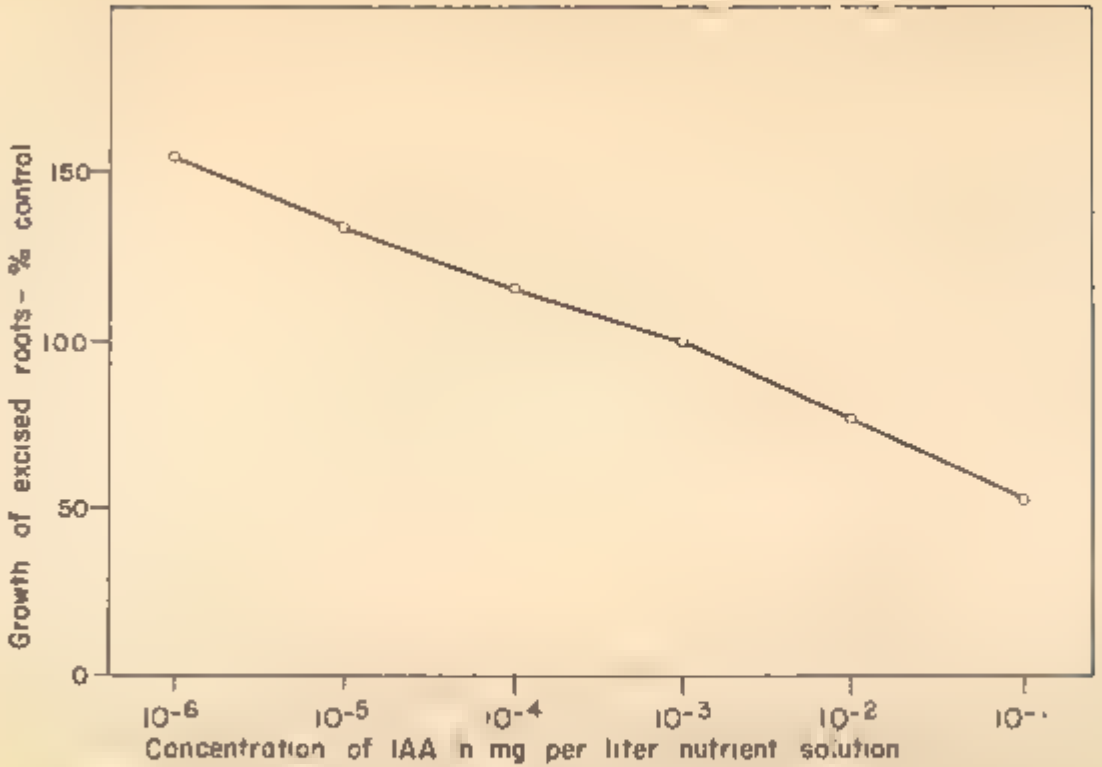

Fig 2 — Mean lengths of excised corn roots after 15 days growth in nutrient solutions containing IAA concentrations from 10^{-6} to 10^{-1} mg per liter nutrient.

TABLE 2

CALCULATION OF LINEAR FORMULAE $y = a + bx$ FOR THE LENGTH OF TISSUE CULTURES (y) AS A FUNCTION OF NUMBER OF DAYS GROWTH (x) BY THE PRINCIPLE OF LEAST SQUARES FOR THE 4 TO 15 DAY

	[redacted]						
Intercept, a	3.3	2.5	2.7	2.4	2.4	2.6	1.3
Rate of Growth, mm/day (Slope, b)	0.20	0.46	0.38	0.30	0.25	0.14	0.13
Standard Deviation of b, (s_b)	0.021	0.007	0.013	0.011	0.009	0.014	0.007
$t_6 = b/s_b$	9.5	65.7	29.2	27.3	27.8	10.0	18.6
Probability, b is not significant	<.001	<.001	<.001	<.001	<.001	<.001	<.001

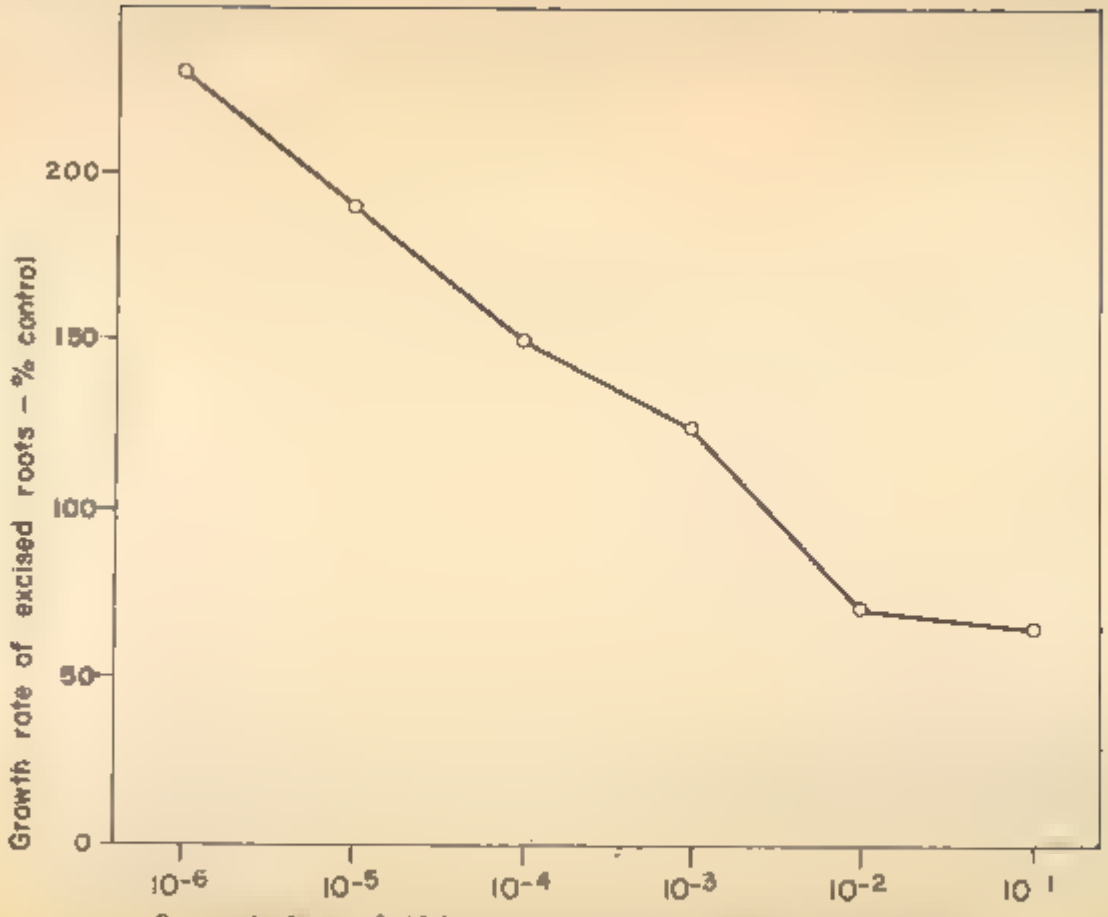

Fig. 3 — Mean growth rates of excised corn roots, for period from 4 to 16 days, in nutrient solutions containing IAA concentrations from 10^{-6} to 10^{-1} mg per liter nutrient

Discussion and Summary

The main question which arises in viewing the results of this experiment is—Why does a high concentration of IAA retard root growth while a low concentration enhances growth? One can speculate that the lower concentration simulates more closely the natural auxin concentration in the growing root. The inhibitory effect on growth of higher auxin concentrations is hypothetically explained by Skoog, et al (10). Auxin is supposed to be active when it functions as the prosthetic group of an enzyme. The prosthetic group of an enzyme effectively bridges the gap between a substrate molecule and the protein part of the enzyme. Relatively high concentrations of the auxin result in auxin molecules taking over all free positions on the protein component of the enzyme. This blocks the reaction which occurs between the protein and substrate molecules and an inhibitory effect, due to reduced enzymatic action, is observed. Inhibition of growth may also result from the production of growth inhibitors when an excess of auxin is present (11). Such inhibitors, with a structure similar to that of auxin, may either occupy positions on the protein component of the enzyme, normally taken over by the active auxin molecules, or react with the substrate. Either course would block the overall reaction which takes place only when the substrate molecules are linked to the protein enzyme by the specific prosthetic group.

The standard deviation of the slope, given in Table 2, of the straight line for C_0 ranges from three to one and one-half times those

for the auxin concentrations. Hence, the fit of the C_0 growth data to a straight line is not as good as that of the auxin-growth data. A qualitative estimate of the goodness of fit may also be obtained from inspection of Figure 4 which shows the calculated lines as compared with the growth data for the different auxin concentrations. The poorest linear fit is observed to be that for the C_0 growth data. This is a faint indication that the indoleacetic acid may also function as a regulator of the rate of formation of meristematic cells and perhaps of cell elongation in the sense that a constant growth rate is maintained. Our data seem to indicate that more constant growth rates are obtained for all the auxin concentrations tested than for the controls.

It is concluded from the observations and statistical analyses:

1. The primary growth of excised corn root tips in nutrient media is inhibited by IAA concentrations of 10^{-1} and 10^{-2} mg per liter nutrient; it is enhanced by IAA concentrations of 10^{-5} and 10^{-6} mg per liter; it is not significantly affected by concentrations of 10^{-3} and 10^{-4} mg per liter. The length of the cultures increases logarithmically with decreasing auxin concentrations.
2. The primary growth rate of the root cultures increases regularly with decreasing auxin concentrations in the nutrient. The highest and lowest growth rates are 0.46 and 0.13 cm per day for IAA concentrations of 10^{-6} and 10^{-1} mg per liter nutrient. These rates are about two and one-half and one-half of the rate observed for cultures grown in nutrient containing no indoleacetic acid.

3. The data faintly indicate that indoleacetic acid, in the range of concentrations used, helps to establish a constant growth rate for excised corn root cultures. This suggests that IAA may assist in the regulation of the rate of formation of meristematic cells or of cell elongation.

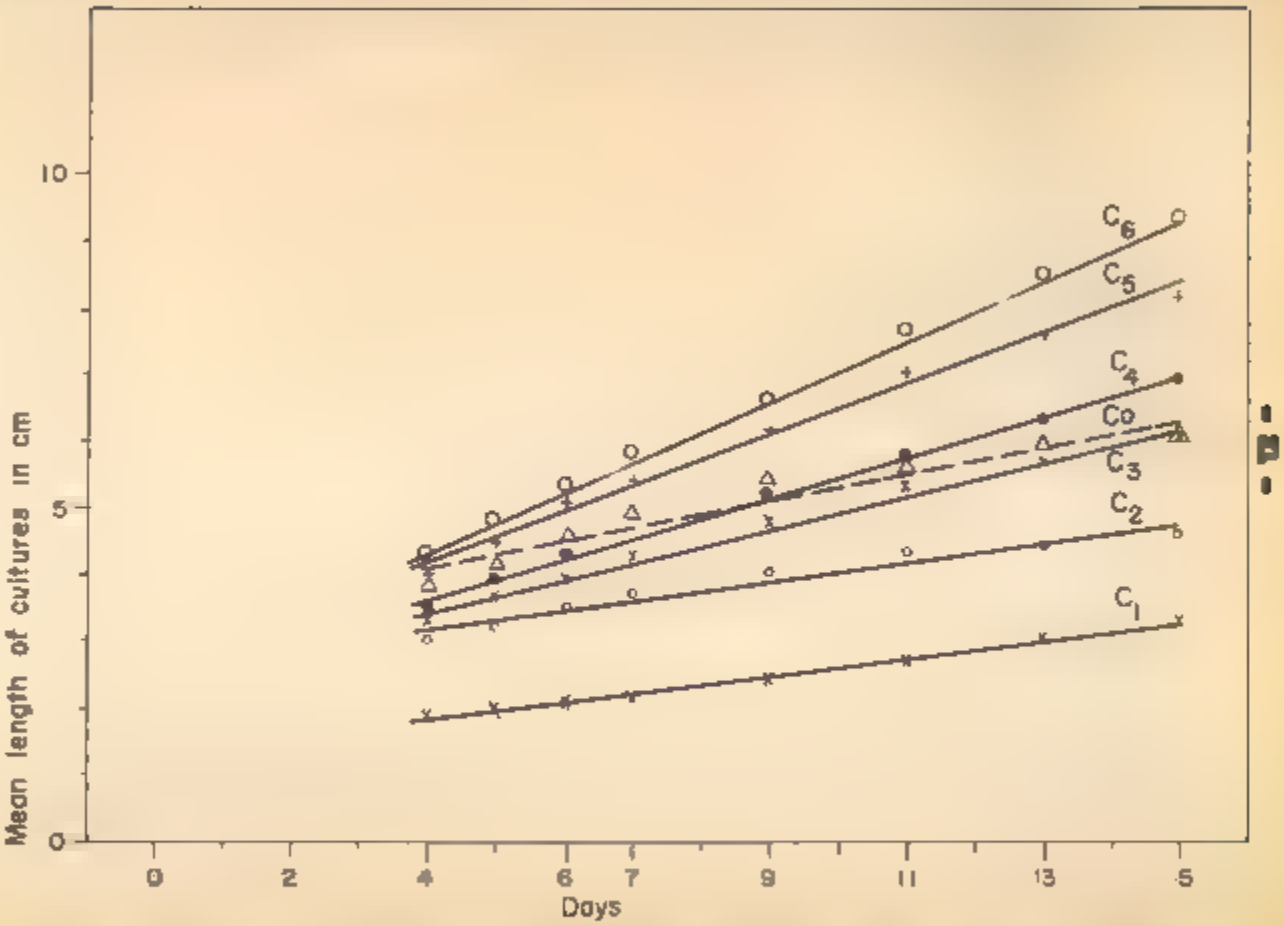

Fig 4 – Linear fits, calculated by the method of least squares, for the lengths of tissue cultures in nutrient media containing IAA concentrations ranging from 0 to 10^{-1} mg per liter

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CHAPTER II

LATERAL ROOT FORMATION ON EXCISED ROOT TIPS AS A FUNCTION OF INDOLEACETIC ACID CONCENTRATION

Introduction

Even at the present time relatively little is known about the processes involved in lateral root formation. Bouillenne and Went (1) found that the application of auxin stimulated adventitious root formation on the stems of Acalypha cuttings. They reasoned that the increased root formation was simply due to the applied hormone not being utilised in the growth of stems. Zimmerman and Hitchcock (2), working with aerial roots of Cissus, discovered that lateral root formation was induced either by applied auxin or root decapitation. They postulated the formation of a factor by the primary root tip which prevented lateral branching but which became inoperative when the tip was interfered with or no longer actively growing.

Thimann (3), working with Avena, verified these results and also found that the removal of only 1 mm root tips resulted in increased lateral root formation. Avena contains large amounts of natural auxin. From this work, Thimann deduced that the principal influence on root branching is exerted by the tip. He surmised that auxin may not be the only factor controlling lateral root formation. Van Overbeek (4), in studying the auxin production in isolated pea roots grown in vitro, found that 10 mm root decapitation resulted in a marked increase in lateral root formation at the basal end of the root. Delarge (5) pointed out also that indoleacetic acid initially caused excess branching in excised root tips of Zea and Triticum grown in sterile culture. However, subsequent retreatment with the auxin caused no further lateral root

formation.

Torrey (6), in a study of lateral root formation in excised pea root tips, found an optimum concentration of 1 mg indoleacetic acid per liter nutrient solution for the growth of branch roots. He states that this concentration produces a maximum and uniformly reproducible response in the number of lateral roots formed. His data on the control roots, grown on agar containing no IAA, show on the average no lateral root formation for the first 15 days of the study. The root tips, initially 3 to 4 mm in length, reached an average length of 7½ mm. Cultures, after 7 days treatment with 1 mg IAA per liter nutrient and subsequent transfer to control media, developed an average of one lateral root after the first week of growth and two lateral roots after the second week of growth. The excised roots, initially 3 to 4 mm long, attained average lengths of 7 and 27 mm for one and two weeks growth. He also deduced that lateral root formation either with or without IAA stimulation is correlated with the elongation of the primary root. Torrey postulates that an unidentified substance other than auxin is necessary for lateral root formation in pea, but that the substance becomes active within the root under the influence of auxin. He also suggests that a balance between naturally produced auxin within the root and lateral root forming substance controls normal lateral root formation in the intact plant.

The purpose of this investigation is to study quantitatively the formation of lateral roots on excised corn root tips grown in sterile nutrient media containing different artificial concentrations of indole-acetic acid with the view of elucidating further the process of lateral

root formation and the role of auxin in the process.

Procedure and Experimental Results

This investigation is ancillary to that of the primary growth of the tissue cultures in sterile nutrient described in Chapter I. The tissue cultures used in the study of primary growth are also used for the observations of lateral root formation. Thus, the preparation of the tissues and the experimental procedures are the same as those described above. The study is made to ascertain whether or not:

1. Auxin affects the formation of lateral roots.
2. Lateral root formation is dependent on the primary growth of the tissue.
3. Auxin enters into the lateral root and primary growth dependences.

In Figure 1, the average numbers of branch roots per tissue culture calculated from groups of ten cultures, each group having different auxin treatments, are plotted against the number of days growth of the cultures. The formation of lateral roots appears to be dependent on auxin treatment. To establish the existence and to find the nature of the dependence of lateral root formation on primary growth, a statistical analysis must be performed on the data. The statistical reduction of data consisting of two observations (x and y) made concomitantly in the course of the same experiment is achieved by the use of regression (dependence) techniques. The simplest relation between x and y from the statistical point of view is that of the straight line $y = a + b(x-\bar{x})$. The technique of linear regression consists of determining the constants a and b and

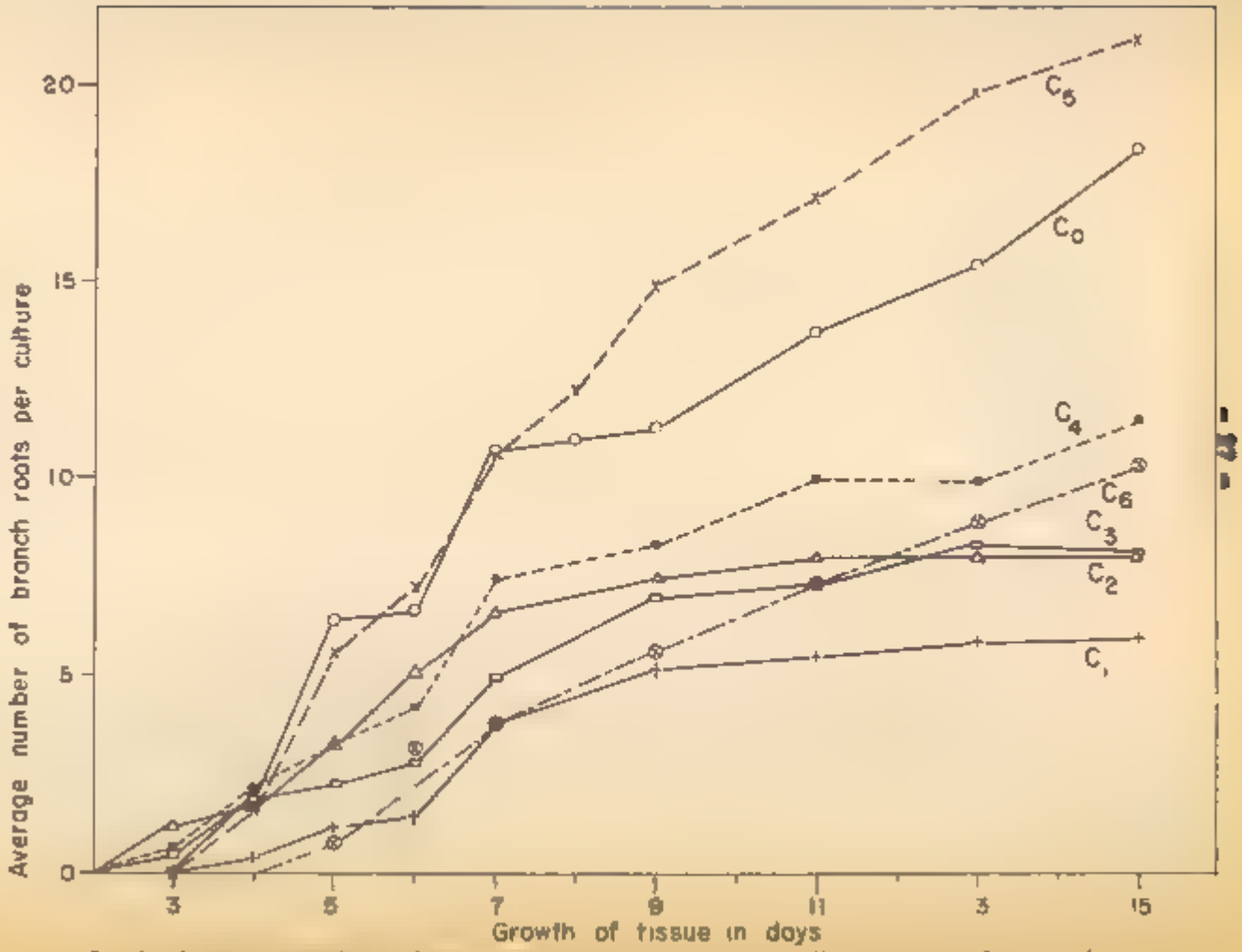

Fig 1 Average number of adventitious roots per tissue culture grown for 15 days in nutrient media containing IAA concentrations from 0 to 10^{-1} mg per liter

also the sampling errors to which they are subject. The constant b is the regression coefficient. If b differs significantly from zero, y shows a definite relation to x, and one activity is dependent on another through some mutual agent. The results obtained for the linear regression of the average number of branch roots per root culture (y) on the average primary length of the excised root (x) are given in Table 1 and shown in Figure 2. The regression coefficients b are all significant. Therefore a definite dependence of branch root formation on the total length of the root exists. A statistical analysis must also reveal whether the regression coefficients for the controls and the auxin treated specimens are homogeneous or whether they are significantly different; i.e., it determines whether or not the auxin is a factor in the dependence of lateral root formation on primary root length. Statistically, the agreement of regression coefficients by an analysis of variance must be tested. A joint regression coefficient b for the pooled data is calculated. From this b, a sum of squares of the joint regression is found and compared with the total sum of squares of the seven separate regression coefficients b_i. The homogeneity between the joint regression and the separate regressions can then be determined by a t-test wherein the difference between the regressions and the error variation are taken into account. In Table 2, the analysis of variance yields a relatively high value of t which corresponds to a very small probability that the various regression coefficients b_i are in agreement. From this, it is concluded that the lateral root formation-primary root length dependence is probably influenced in some way by the presence of auxin.

TABLE 1

SUMMARY OF THE LINEAR REGRESSION OF THE NUMBER OF BRANCH ROOTS y ON THE PRIMARY LENGTH OF THE ROOT CULTURES x

Conc. IAA, gm per Liter	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
Avg. No. branch roots per tissue, a	10.60	5.73	12.20	6.38	4.80	5.50	3.69
Standard Deviation of a, s_a	0.41	0.18	0.48	0.31	0.25	0.26	0.42
Regression Coeff., b	6.39	1.97	4.64	2.94	2.67	4.01	4.06
Standard Deviation of b, s_b	0.54	0.11	0.30	0.26	0.25	0.42	0.88
t	11.9	17.5	15.4	11.5	10.8	9.67	4.60
Probability that y does not depend on x	<.001	<.001	<.001	<.001	<.001	<.001	.01-.001

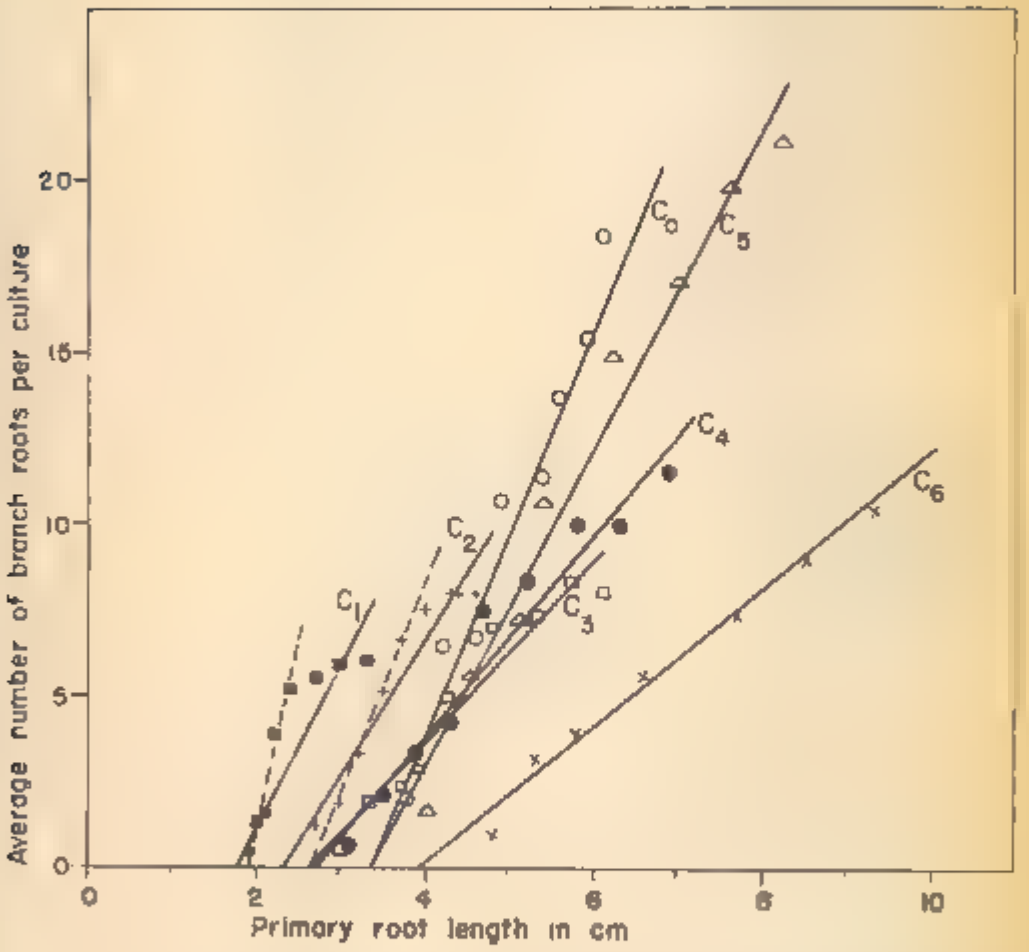

Fig. 2 – Linear fits to the average number of lateral roots per tissue as a function of primary root length for concentrations of IAA of 10^{-6} to 10^{-1} mg per liter nutrient.

[redacted]

ANALYSIS OF VARIANCE TO TEST JOINTLY THE HOMOGENEITY OF THE REGRESSION COEFFICIENTS b OBTAINED FOR ALL AUKIN CONCENTRATIONS

Item	Sum of Squares	N	Mean Square	b	Probability
Joint Regression	759.965	1	759.965		
[redacted] Regression	111.992	1	111.992	11.0	<.001 (very small)
Error	40.76	44	0.926		

Table 1 shows that $a = \bar{y}$, the mean number of branch roots, differs for various auxin treatments. These values of $\bar{y}$ are shown in Figure 3 as percentages of the control, $\bar{y}_c$. The maximum of the curve corresponds to a concentration of 10^{-5} mg IAA per liter nutrient. The data indicate this to be a rather sharp inversion concentration. The values of the regression coefficients b constitute the best estimates of the number of branch roots per unit length of the root. The control or untreated culture has the greatest slope; the culture treated with 10^{-5} mg IAA per liter nutrient, the next greatest slope. The slopes corresponding to treatments of the other concentrations of IAA follow no pattern other than a general decrease of the slope with decreasing auxin concentration; the slopes of the control and the concentration of 10^{-5} mg per liter do not conform to this trend. Since the fit of the data to straight lines is not always very good, these slopes may not justifiably be considered as rates of branch root formation per unit of total culture length. The slopes, in these cases indicate only that the number of branch roots is related to the primary length of the tissue culture, and that the degree of the relationship (viz. the inhomogenity of the slopes b_i) differs in the presence of various concentrations of auxin.

Discussion and Summary

It is interesting to note that the concentration of indoleacetic acid in the nutrient producing the greatest enhancement of primary growth of the root cultures does not stimulate the formation of lateral roots. In fact, the action of this concentration, 10^{-6} mg per liter, of

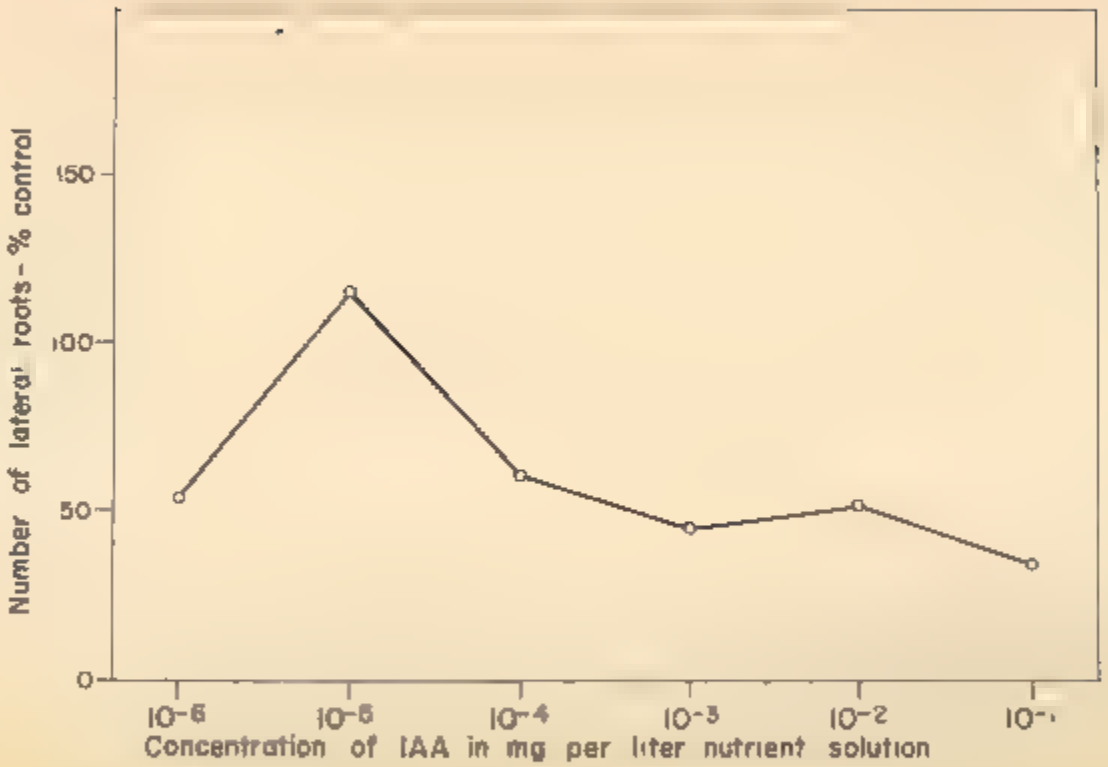

Fig. 3 Average number of lateral roots formed on excised corn roots grown in nutrient media containing IAA concentrations from 10^{-6} to 10^{-1} mg per liter nutrient

indoleacetic acid in the formation of lateral roots is hardly distinguishable from that of auxin concentrations which inhibit primary root growth. However, on account of the strong dependence of branch root formation on the primary length of the tissue cultures, it is not valid to draw conclusions about the role auxin plays in lateral branching without consideration of the lengths of the tissue cultures. Inspection of Figure 1 alone would lead to a conclusion that the concentration of 10^{-5} mg per liter stimulates lateral root growth. Inclusion of a study of Figure 2 suggests that the appearance of more branch roots per tissue culture in the presence of 10^{-5} mg per liter IAA could more likely be attributed to a greater primary growth of the C_5 tissues than of the C_0 tissues rather than to an enhancement of lateral root formation by this concentration of auxin. The C_0 curve of Figure 2 rises more rapidly than the C_5 curve, and the control tissue cultures may be considered to form more branch roots per unit total length of tissue than the tissues grown in auxin concentrations of 10^{-3} to 10^{-6} mg per liter nutrient.

Several workers have pointed out that indoleacetic acid initially caused excess branching in excised root tips. If the initial rise only of the C_1 and C_2 curves in Figure 2 are considered, the slopes of these lines would be steeper than that of the control curve. Now, comparison of these slopes with those in Chapter I for the primary growth rates corresponding to various concentrations of IAA suggests that the effect of auxin on primary growth and branch root formation is inverse. When the primary growth is speeded up, the rate of production of lateral roots is slowed down. When primary growth is inhibited, the initial

rate of appearance of lateral roots is enhanced. The determination of whether the growth activity is greater in the direction of total length or in the direction of branching seems to hinge on the concentration of auxin present.

Torrey's investigation is more along the lines of the present study than other previous work on lateral root formation and is best suited for a comparison. The high concentration of 1 mg IAA per liter nutrient which he found optimum for lateral branching in excised pea roots is greater than the highest concentration used in this investigation. But the highest initial rate of lateral root formation found here corresponds to the greatest concentration of IAA. Hence the comparable observations in the two investigations are not in disagreement.

This investigation reveals that the growth hormone auxin seems to take part in the growth activity not only as a regulator of the rate of growth but also as a regulator of the extent of branching. Very low concentrations of 10^{-5} and 10^{-6} mg IAA per liter enhance the growth in length of the tissue cultures while the higher concentrations of 10^{-1} and 10^{-2} mg IAA per liter stimulate lateral branching.

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CHAPTER III

THE RESPIRATION OF EXCISED ROOTS AS A FUNCTION OF INDOLEACETIC ACID CONCENTRATION

Introduction

As a general rule, respiration rates are greatest in meristematic tissues, such as growing root tips, since large quantities of carbohydrates are oxidized in these regions of dividing cells (1). Beatty has shown a direct relation between the mitotic rate and rate of oxygen consumption in young leaves (2). Moreover, regions of rapid cell elongation are centers of high respiratory activity. In such regions the rate of respiration is not much less than the rate in regions of cell division.

Since experiments indicate that auxins take part in the carbohydrate or organic acid metabolism of plants, it is reasonable to expect that enhancement or inhibition of primary growth of excised roots by indoleacetic acid would also be reflected in an increased or decreased respiration rate of the roots. In fact, it has been indicated that many different kinds of tissue immediately respire at an increased rate through the application of auxin in concentrations suitable for growth stimulation (3, 4). Even though auxin appears to take part in the respiratory process, the nature of the relation of auxin and respiration has not been found.

The purpose of this experiment is to determine whether or not the respiration of excised corn roots in sterile nutrient media is affected by several different concentrations of indoleacetic acid that have been found to inhibit as well as to enhance primary root growth.

Experimental Procedure and Results

The preparation of the tissue cultures is the same as that for the

previous experiments. The respiration rates of cultures grown for one day at 20° C in sterile nutrient media containing five different concentrations of indoleacetic acid, ranging from 10^{-5} to 10^{-1} mg per liter, are compared with that of the controls grown in nutrient containing no artificial supply of indoleacetic acid. For the respiration observations, the cultures, in groups of four, are transferred to 15 ml Warburg flasks containing 2 ml of the nutrient solution in which they were grown and 0.2 ml of 20% KOH in the center well. The oxygen uptake of the tissues is observed at ten minute intervals for the first half hour and then at half hour intervals for about four hours.

The observations of oxygen consumption are reduced to rates of respiration by the technique of linear regressions. The four tissue cultures in one flask are not likely to be the same size as four tissue cultures in another flask. The most accurate estimate of the sizes of the tissues is their dry weight. The tissues are dried after the oxygen uptakes are measured and the groups of four tissues are then weighed. The concomitant measurements of dry weight are of importance in interpreting the results of the observations of oxygen consumption. For any difference in respiration rate between cultures from two different media could perhaps be ascribed to weight discrepancies. To take account of the weight differences, either some arbitrary correction of the oxygen consumption can be made to allow for variations in weight, or the data can be used to supply their own correction. The most obvious and most widely used arbitrary correction for weight difference is that of using

the oxygen consumption per unit weight. The respiration rates, their standard deviations, and the rates, after the a priori correction for weight is made, are tabulated in Table 1. The microliters of oxygen consumed per hour per milligram dry weight of the cultures are plotted for the various concentrations of IAA in their nutrient solution in Figure 1. No relation of respiration rate to auxin concentration is apparent.

The a priori correction for weight is questionable. When the uncorrected respiration rates are plotted against dry weight in Figure 2, it is apparent that no simple increase of respiration with increasing dry weight is observed. Thus the results of a comparison of respiration rates which utilizes the arbitrary correction for dry weight are not conclusive.

The correction for weight discrepancies may be supplied by the data of the experiment. Without calculating the actual regression formulae of the respiration on weight, the analysis of covariance is adopted to perform the test of significance of the differences between the respiration rates of the tissue cultures grown in nutrient containing indoleacetic acid and of those grown in nutrient without IAA. The details of the analyses and the results of the significance tests are given in Table 2. Even when the objectionable arbitrary correction for weight differences is eliminated, no significant difference in the respiration rates, which can be attributed to auxin, is found.

[redacted] I

THE RESPIRATION RATES OF TISSUE CULTURES AFTER 24 HOURS IN [redacted] DIFFERENT VARIOUS AMOUNTS OF [redacted]

IAA conc.(mg/liter)		0	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
[redacted] No. 1	$\mu l O_2/hr$	38.95	30.48	48.31	28.17	41.14	28.99
	Standard Deviation	.29	.31	.70	.29	.43	.42
	Dry Weight (mg)	5.00	3.70	4.00	2.95	3.80	3.20
	$\mu l O_2/hr/mg$	7.79	8.24	12.1	9.55	10.8	9.03
Run No. 2	$\mu l O_2/hr$	30.11	40.14	36.49	31.43	42.71	26.14
	Standard Deviation	.43	.40	.35	.25	.28	.46
	Dry Weight (mg)	2.85	3.25	2.65	3.35	3.75	1.95
	$\mu l O_2/hr/mg$	10.6	12.4	13.8	9.38	11.4	13.4
Run No. 3	$\mu l O_2/hr$	22.88	25.65	19.42	20.89	33.71	[redacted]
	Standard Deviation	.32	.13	.27	.37	.58	.42
	Dry Weight (mg)	2.60	3.05	2.25	2.65	3.15	1.95
	$\mu l O_2/hr/mg$	8.80	8.41	8.63	7.88	10.7	9.99
Run No. 4	$\mu l O_2/hr$	15.72	14.78	18.82	14.99	21.25	20.06
	Standard Deviation	.12	.21	.30	.27	.20	.33
	Dry Weight (mg)	0.7	0.4	1.4	1.4	1.8	1.6
	$\mu l O_2/hr/mg$	22.5	37.0	13.4	10.7	11.8	12.5

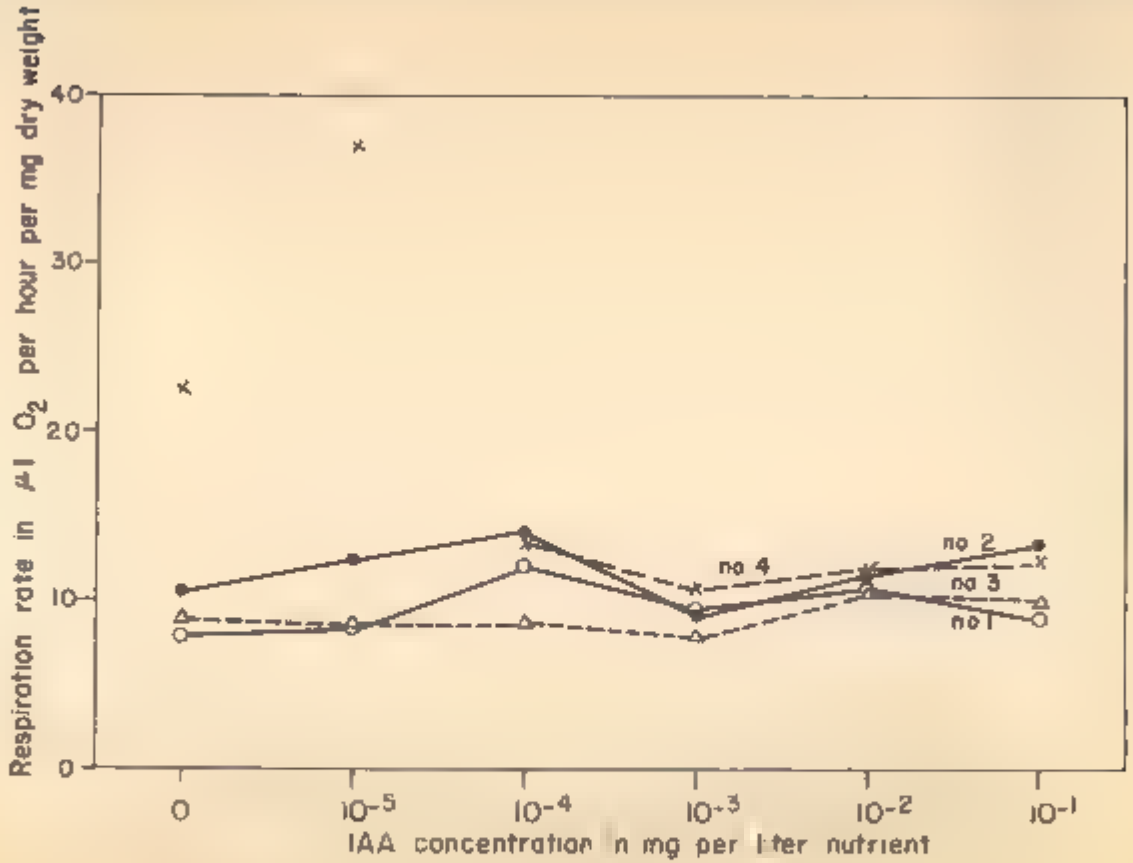

Fig. 1 – Respiration of excised corn roots cultured in nutrient media containing IAA concentrations of 10^{-5} to 10^{-1} mg per liter

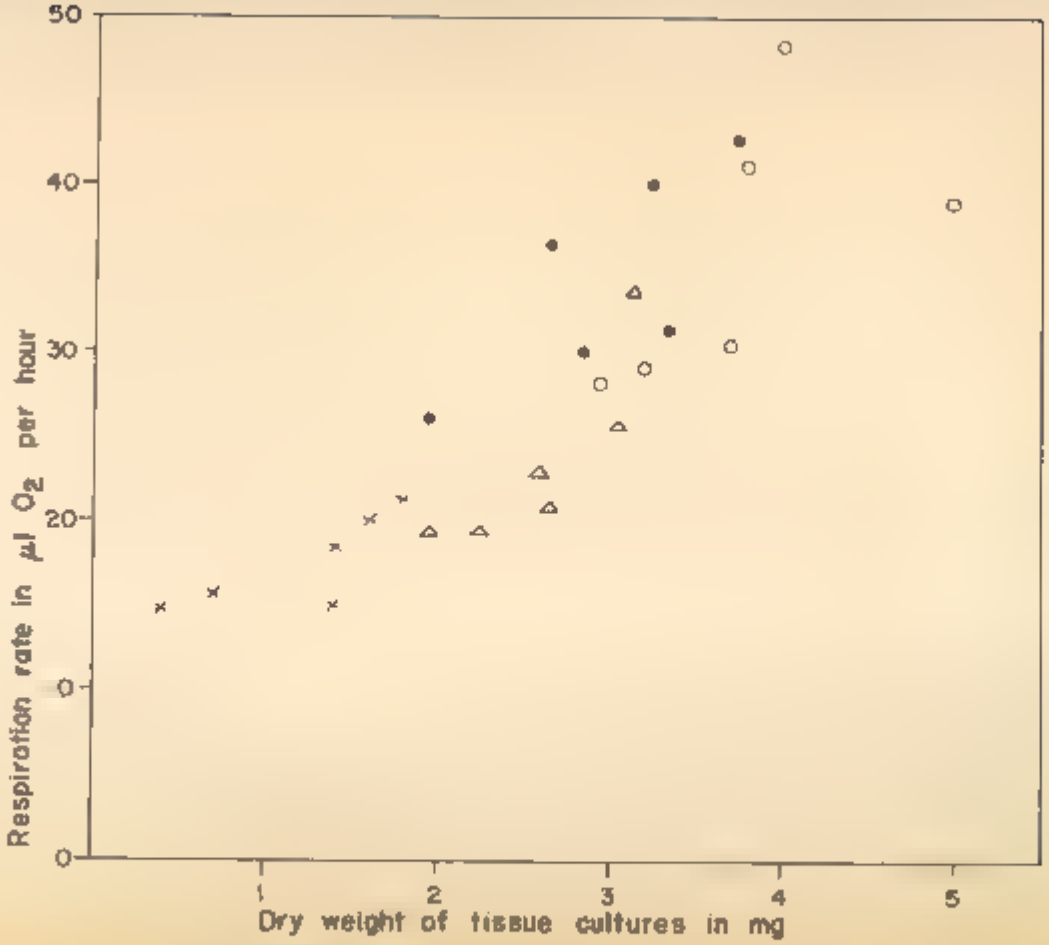

Fig 2 – Respiration rates of tissue cultures as a function of their dry weight

TABLE I

ANALYSES OF COVARIANCE AND VARIANCE OF RESPIRATION RATES OF EXCISED CORN ROOTS CULTURED IN NUTRIENT MEDIA CONTAINING DIFFERENT IAA CONCENTRATIONS

			Analysis of Covariance		
Series	Item	N	x^2	xy	y^2
C_4 and C_5	Between Series	1	0.0703	- 0.318	1.43
	Within Series	6	15.9669	89.60	630.25
	Total	7	16.0372	89.28	631.68
C_3 and C_4	Between Series	1	0.0903	- 1.63	29.57
	Within Series	6	12.8144	94.11	908.65
	Total	7	12.9047	92.48	938.22
C_3 and C_3	Between Series	1	0.0800	1.22	18.55
	Within Series	6	11.4188	68.43	460.02
	Total	7	11.4988	69.65	478.57
C_3 and C_2	Between Series	1	0.8278	5.25	121.29
	Within Series	6	11.8944	78.10	584.16
	Total	7	12.1222	83.35	705.45
C_3 and C_1	Between Series	1	0.7503	3.98	21.09
	Within Series	6	10.7744	58.87	361.44
	Total	7	11.5247	62.85	382.73

TABLE 8—Continued

Correction for Regression of y on x	Analysis of Variance of y After Correction				
	DF	Sum of Squares	Mean Square	F	Probability
	1	7.20	7.20	0.53	.70-.50
502.80	5	127.45	25.49		
497.03	6	134.65			
	1	57.97	57.97	1.16	.30
691.15	5	217.50	43.50		
662.75	6	275.47			
	1	6.72	6.72	0.64	.50-.30
412.48	5	47.54	9.51		
424.31	6	54.26			
	1	61.00	61.00	2.06	.10
512.41	5	71.35	14.27		
573.10	6	132.35			
	1	0	0	0	1.00
321.66	5	39.98			
342.75	6	39.98			

Discussion and Summary

Previously, it has been found that the amount of sugar in carrot tissues was less, when the nutrient contained 10^{-6} parts of indoleacetic acid, than the sugar content of tissues grown in nutrient containing no auxin. From this, Goris (5, 6) concluded that indoleacetic acid enhanced the proliferation of the tissues up to a certain limit and at the same time caused the excess use of the carbohydrates. In the light of this work, it is surprising that no difference in the respiration rates of excised corn roots is detectable when the tissues are cultivated in different concentrations of indoleacetic acid which have been found not only to inhibit but also to enhance primary growth.

The inhibition of both growth and respiration of coleoptile sections has been reported (7, 8). A respiration inhibition of 5% was obtained for a 7.2×10^{-3} molar concentration of fluoride. However, Benner and Thimann (9) noted that such a concentration is well above that necessary to produce growth inhibition and would be highly toxic to the coleoptile. They also found that at a fluoride concentration sufficient only to produce 50% growth inhibition, there is no appreciable inhibition of respiration in the coleoptile. This work was also verified in the case of pea stems (10).

On the basis of the above work on coleoptiles and pea stems, although a comparison is not strictly valid, it may be concluded, from the experimental results obtained with excised corn roots, that either the mechanism of action of auxin on the processes of growth and respiration

is somewhat different or that the respiration process which controls growth, being undetectably small, is responsible for only a minute fraction of the total oxygen consumption of the root.

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AS A FUNCTION OF X-RAY DOSE

Introduction

The results of previous investigations concerned with the effect of X-rays on respiration are not in complete accord. Even though it would be expected that the profound effects of X-radiations on living systems, evident in many different ways, would also be reflected in the gaseous exchange which is characteristic of the overall metabolism of the system, many investigators report that no effect on the respiration is evident. Boell (1) working with grasshopper eggs and embryos, found that their respiration was completely normal for as long as 12 days after X-ray doses up to 5000 r. Tahmisian (2, 3) irradiated grasshopper eggs with dosages of X-rays ranging from 25,000 to 200,000 r. Depression of respiration was noted only for the strongest dosages. On the other hand, Tahmisian and Barron (4) have reported that the respiration of the eggs is significantly depressed by dosages as small as 10 r. This inhibition of respiration lasts only a short time though and the respiration resumes a normal rate within five hours. Such an inhibition could have been easily overlooked in the above work since in those experiments respiratory measurements were not begun immediately after the eggs were irradiated. Chesley (5) irradiated sea urchin eggs with X-ray dosages up to 43,000 r and reported that the respiration was unaffected. Barron, et al, (6) investigated the effects of X-rays on the respiration of sea urchin sperm suspensions. They found that the oxygen uptake was reduced by 10% with 100 r and decreased to 66% of the control for increasing dosse up to

20,000 r. Barton (7), in a study of the cell multiplication and the respiration of X-irradiated bacteria, found that it was possible to inhibit respiration without altering cell multiplication.

It is possible that the inhibiting effects of radiation on respiration have not been detected to any degree because the organisms selected for previous experimentation have, in general, rather complex cellular structures. In different localities of these more complex living systems, respiration may proceed at vastly different rates after irradiation and yet the overall metabolic activity, as reflected in respiration, may be unaffected. Therefore, it would be desirable to study the effects of radiation on systems, which have a relatively simple cellular structure, such as root cultures to determine whether or not there is any change in their respiration with radiation.

The purpose of this experiment is to determine whether or not the respiration of excised corn roots grown in sterile nutrient media is affected by X-rays.

Experimental Procedure and Results

The preliminary preparation of the excised corn roots is the same as that outlined previously. Immediately after excision of the root tips, four each of the excised roots are given X-ray doses ranging from 100 to 1400 r. The X-ray unit is operated at 65,000 volts and the radiation is filtered through $\frac{1}{2}$ mm of Al. The root tips are then transferred to 125 ml Erlenmeyer flasks containing 50 ml of White's nutrient solution augmented by 10^{-5} mg per liter indoleacetic acid. The cultures

are incubated for 24 hours at 30° C.

The irradiated cultures in groups of four are transferred to 15 ml Warburg flasks containing 2 ml of the nutrient solution in which each group is grown and 0.2 ml of 20% KOH in the center well. The oxygen uptake of the cultures is measured by use of standard Warburg techniques (8) at ten minute intervals for the first half hour and then at half hour intervals for about four hours. The Warburg bath is operated at 31° C. Then, each group of tissues is dried for about 20 hours at 300° C and their dry weights measured. Three separate Warburg runs are made to measure the oxygen consumption of tissues having X-ray doses from 100 to 1400 r.

Figure 1 shows the mean respiration rates for the three Warburg runs in μl O_2 per mg dry weight per hour as a function of the X-ray dose. Table 1 is a summary of the respiration data for the irradiated tissue cultures. An analysis of the linear regression relating ln Q_{O_2} to the X-ray dose must be carried through to determine whether or not the respiration rate decreases exponentially with increasing radiation (9). The data are arranged in sets according to the three different Warburg runs. The results of the calculations tabulated in Table 2 are given by the analysis of variance of the three series of observations. The first t test shows no significant difference between the different sets of observations. That is, the three separate Warburg runs are in good agreement with each other and form a homogeneous set of observations. The second t test gives the significance of the linear dependence of the ln respiration on the different X-ray doses. A probability of 0.05 here

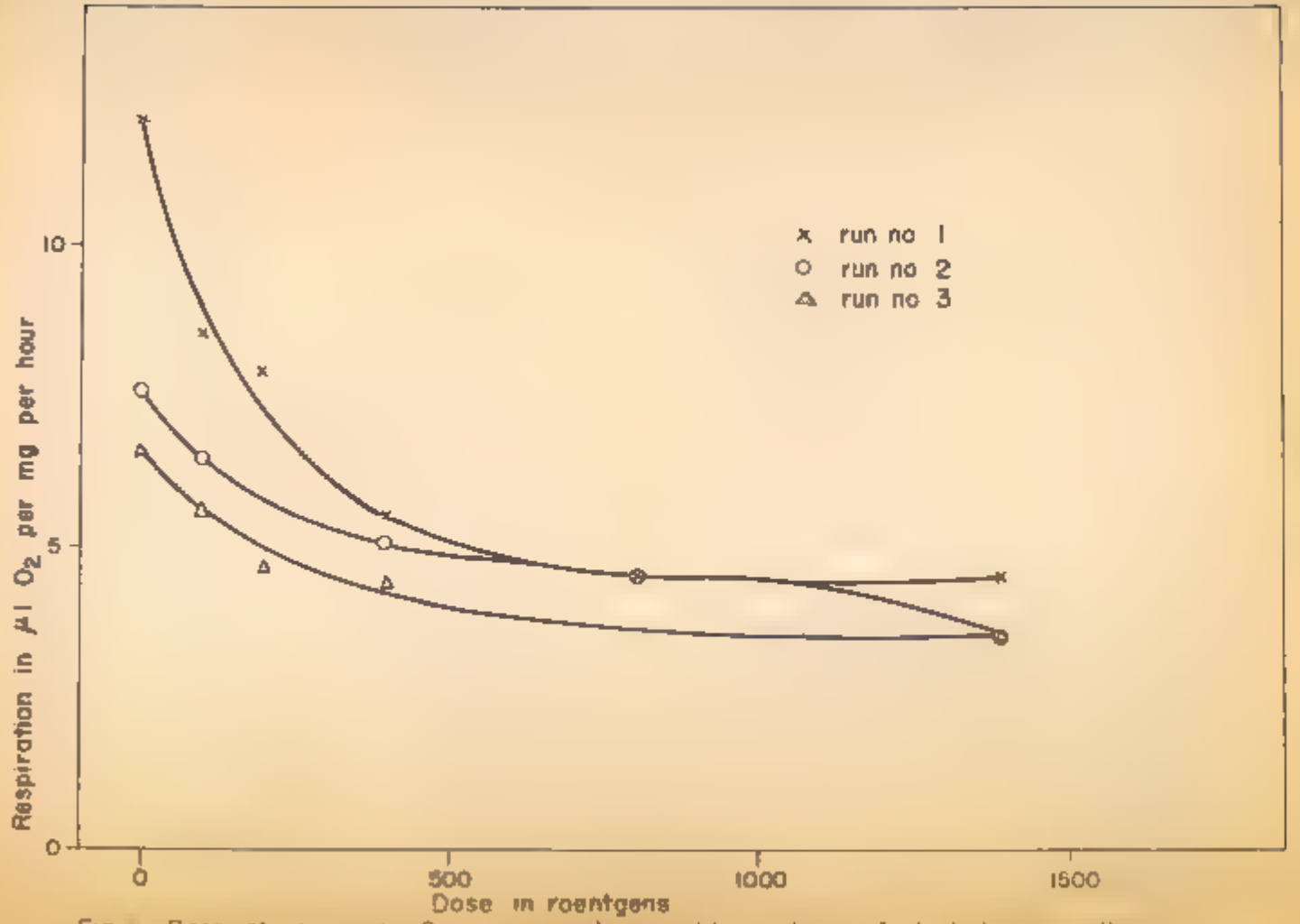

Fig [illegible] - Respiration in µ O_2 per mg dry weight per hour of plant tissue cultures having X-ray doses ranging from 0 to 1400 roentgens

SUMMARY OF RESPIRATION RATES OF IRRADIATED TISSUE CULTURES

Date (1952)	Dose	Rate (mm/hr)	Flask Const at 31° C (μlO_2/mm)	Rate (μlO_2/hr)	Dry Wgt tissue (mg)	Rate (μlO_2/mg/hr)	Vessel No.	Notes
July 25	0r	9	1.72	15.5	1.3	11.9	8	31° C
	100r	12	1.57	18.8	2.2	8.5	9	
	200r	12	1.53	18.4	2.3	8.0	10	
	400r	9	1.70	15.3	2.8	5.5	11	
	800r	8	1.66	13.3	2.9	4.6	12	
	1400r	9	1.60	12.6	2.8	4.5	13	
Aug.4 (run No.1)	0r	8	1.72	13.8	2.1	6.6	8	31° C
	100r	8	1.34	10.7	1.9	5.6	2	
	200r	6	1.23	7.5	1.6	4.7	3	
	400r	6	1.52	9.1	2.0	4.5	5	
	800r	-	1.45	—	—	(3.6)	6	(contaminated)
	1400r	6	1.51	9.1	2.7	3.4	7	
Aug.4 (run No.2)	0r	7	1.41	9.9	1.3	7.6	1	31° C
	100r	9	1.57	14.1	2.2	6.4	9	
	200r	9	1.53	13.8	2.4	5.7	10	
	400r	6	1.70	10.2	2.0	5.1	11	
	800r	8	1.66	13.3	2.9	4.6	12	
	1400r	8	1.60	11.2	3.2	3.5	13	

TABLE 2

[redacted]

Item	Sum of Squares	df	Mean Square	Variance Ratio	%	Probability
Sets	0.435082	2	0.217531	22.389946	4.731	0.05
Regression	1.129167	1	1.129167	116.217270	10.780	0.05
Remainder	0.215052	4	0.053763	5.533450	2.353	0.10
Interaction	0.097157	10	0.009716	—		
Error	0.000000	0	—			
Total	1.876438	17				

indicates that the decrease of respiration with increasing dose is probably exponential. The last t test shows that the probability of a fit as bad or worse with the hypothesis of linear dependence is about one in ten. From the latter two tests it is concluded that the respiration rates probably decrease exponentially with increasing X-ray dose. The ln respiration decreases on the average by 0.05 for each 100 r.

To determine whether the respiration of irradiated tissue cultures is truly different than that of unirradiated tissues or the observed decrease in the respiration rate is merely due to chance, the data are divided into two groups: controls or unirradiated tissues and those having the higher doses of X-rays. The data given in Table 3 are then subjected to analyses of covariance and variance. The analysis of covariance is the simultaneous analysis of two variables, the respiration rates and the dry weights. In this case it was used to supply the correction for the dependence of the respiration rate on the dry weight. The analysis of variance is to compare the variation of respiration between the two series with the error variation.

The design of the experiment called for these particular analyses. The concomitant measurements of dry weights must be incorporated into the statistical analysis. It is logical to assume respiration to be dependent on the size of the tissues but the relationship is not known. In the first calculation the oxygen consumption in μl per hour is used and the data are allowed to supply their own correction for the dependence of respiration on weight. The analysis of variance gives a probability of

TABLE 3

OXYGEN CONSUMPTION OF PLANT TISSUE CULTURES GROWN IN NUTRIENT CONTAINING 10^{-5} MG AUXIN PER LITER

Series	Dry Weight in mg	O_2 used $\mu l O_2/hr$	O_2 used $\mu l O_2/mg/hr$
	3.7	29.4	7.9
	3.2	38.4	12.0
	3.0	25.2	8.4
Not	0.4	14.8	37.0
Irradiated	1.3	15.5	11.9
	1.3	9.9	7.6
	2.1	13.8	6.6
A	15.0	147.0	91.4
	2.9	13.3	4.6
	2.8	12.6	4.5
	2.8	9.1	4.5
Irradiated	2.7	9.1	3.4
	2.0	10.2	5.1
	2.9	13.9	4.8
	3.2	11.2	3.5
B	18.5	78.8	30.2
Total	33.5	225.8	121.6

less than 0.001 which refutes the null hypothesis that equal weight tissues respire at the same rate whether they are irradiated or not. Thus the respiration rates of the unirradiated and irradiated tissues are truly different. The results of the calculation are tabulated in Table 4. The second calculation is carried through in the same way. Here the respiration rates are arbitrarily corrected for tissue weights. The analysis of covariance, Table 5, shows that the null hypothesis can be accepted once in twenty times. This, in itself, is insufficient evidence to show that the a priori correction of respiration rate is not justified. However, when the results of the previous calculation are reviewed it is apparent that the arbitrary correction has not completely allowed for the weight differences even though the respiration rates, arbitrarily corrected for dry weight, of the two series are found to be significantly different. In the case of observations of the above concomitant type, this study indicates that the analysis of covariance should be used and the experiment allowed to supply its own correction since a priori corrections may give misleading values of significance.

[redacted]

It is shown in the next section of this report that X-rays have an inhibiting effect on the growth of the tissue cultures. The total investigation reveals:

1. X-rays inhibit growth and respiration of excised corn roots.
2. Higher concentrations of indoleacetic acid inhibit the growth but not the respiration of excised corn roots.

TABLE 4

ANALYSES OF COVARIANCE AND VARIANCE OF RESPIRATION RATES IN $\mu l O_2$ PER HOUR FOR UNIRRADIATED AND IRRADIATED TISSUES

[redacted]

Item	N	x^2	xy	y^2	Correction for Regression of Y on X
Between Series	1	0.875	-17.050	332.231	
Within Series	12	10.034	63.353	655.278	400.000
Total	13	10.909	46.303	987.509	196.532

ANALYSIS OF VARIANCE OF y AFTER CORRECTION

Item	N	Sum of Squares	Mean Square	t	Probability
Between Series	1	535.699	535.699	4.805	<0.001
Within Series	11	255.278	23.207		
Total	12	790.977			

TABLE 5

ANALYSIS OF COVARIANCE OF RESPIRATION RATES IN $\mu l O_2$ PER MG DRY WEIGHT PER HOUR OF UNIRRADIATED AND IRRADIATED TISSUES

Item	N	x^2	xy	y^2	b_{12}	Probability
[illegible]	1	0.875	-15.300	267.531	2.150	0.05
[illegible]	12	10.034	-49.971	697.826		
Total	13	10.909	-65.271	965.357		

Thus, it follows that the action of radiation on the processes of growth and respiration is different than that of indoleacetic acid.

Skoog (10) has found the auxin level in plants to be depressed after they have been exposed to radiation. The suggestion has been advanced that radiation suppresses galls by depressing the auxin level (11). The above results do not support this proposal since even a large depression in auxin level by X-rays would not lead to the observed significant inhibitions of both growth and respiration. It is possible that the auxin level in the cultures is depressed by the radiation but it must be inferred that the observed biological effects of radiation are not due solely to reduced auxin activity.

One of the factors affecting respiration measurements is the temperature at which the oxygen uptake is observed (12). It is possible that the decrease in respiration of the irradiated cultures is merely due to the generation of heat in the body of the culture by the ionizing radiation. However, other possible actions of radiations should not be disregarded. There are: (1) the inactivation of enzymes, (2) the formation of activated water and cell poisons, and (3) the direct action of the radiation.

The present study may throw some light on the "inactivation of enzymes" theory of radiation damage. The root cultures grown in a nutrient with a concentration of 10^{-1} mg indoleacetic acid per liter obtained in two weeks an average length which was 54% of the length of the control. The root cultures which were irradiated with 1400 r reached an average length which was 46% of the length of the control. Roughly then, the two

mechanisms bringing about the inhibition may be compared. In Chapter II it is mentioned that auxin may function as the prosthetic group of an enzyme and the presence of an excess amount of the hormone may inhibit growth by a reduction of the enzymatic action. In Chapter III it is found that auxin in this concentration does not depress the respiration rate. If this theory of the action of auxin is accepted, then the theory of radiation damage by inactivation of the auxin-enzymes is open to question since the respiration rate is lowered when the tissues are irradiated. The theory of inactivation of enzymes by an excess of auxin and the theory of inactivation of enzymes by radiation cannot both be accepted. The radiation damage appears to be more drastic than the inhibitory effects of an excess amount of auxin. The concentration of indoleacetic acid which inhibits the primary growth does not damage the activity of lateral branching while the 1400 r dose of X-radiation completely stops the formation of branch roots.

From the observations and the statistical analyses it may be [redacted]

1. The respiration rate of excised corn roots grown in sterile nutrient media is significantly lowered by X-ray doses up to 1400 r.
2. The respiration rate of the cultures most probably decreases exponentially with increasing X-ray dose.

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THE GROWTH OF EXCISED ROOTS AS A

[redacted]

Introduction

The inhibitory effect of X-radiations on the growth of living systems is well known. However, an investigation of the effect of X-radiation on the growth of the excised corn root cultures is of value to indicate to what extent their growth is inhibited by the doses of radiation of the magnitudes necessary to inhibit respiration of the tissue.

Experimental Procedure and Results

Excision of the roots, exposure to radiation, and transfer to a nutrient solution with IAA concentration of 10^{-5} mg per liter are carried out in the same manner as for the preceding experiment. Groups of five tissue cultures subjected to various dosages of X-radiation are grown for a two week period. The mean values of the measurements of length and number of lateral roots during the two weeks are given in Figures 1 and 2.

Discussion and Summary

The growth of the tissue cultures becomes less as the dosage of radiation increases. This result is in complete agreement with past experiments. Fifteen days after irradiation with 1400 r, the lengths of the tissue cultures are only 1/6 of the lengths of the controls. The same amount of X-radiation depressed the respiration rate one day after irradiation to one-third the rate of the control tissue cultures.

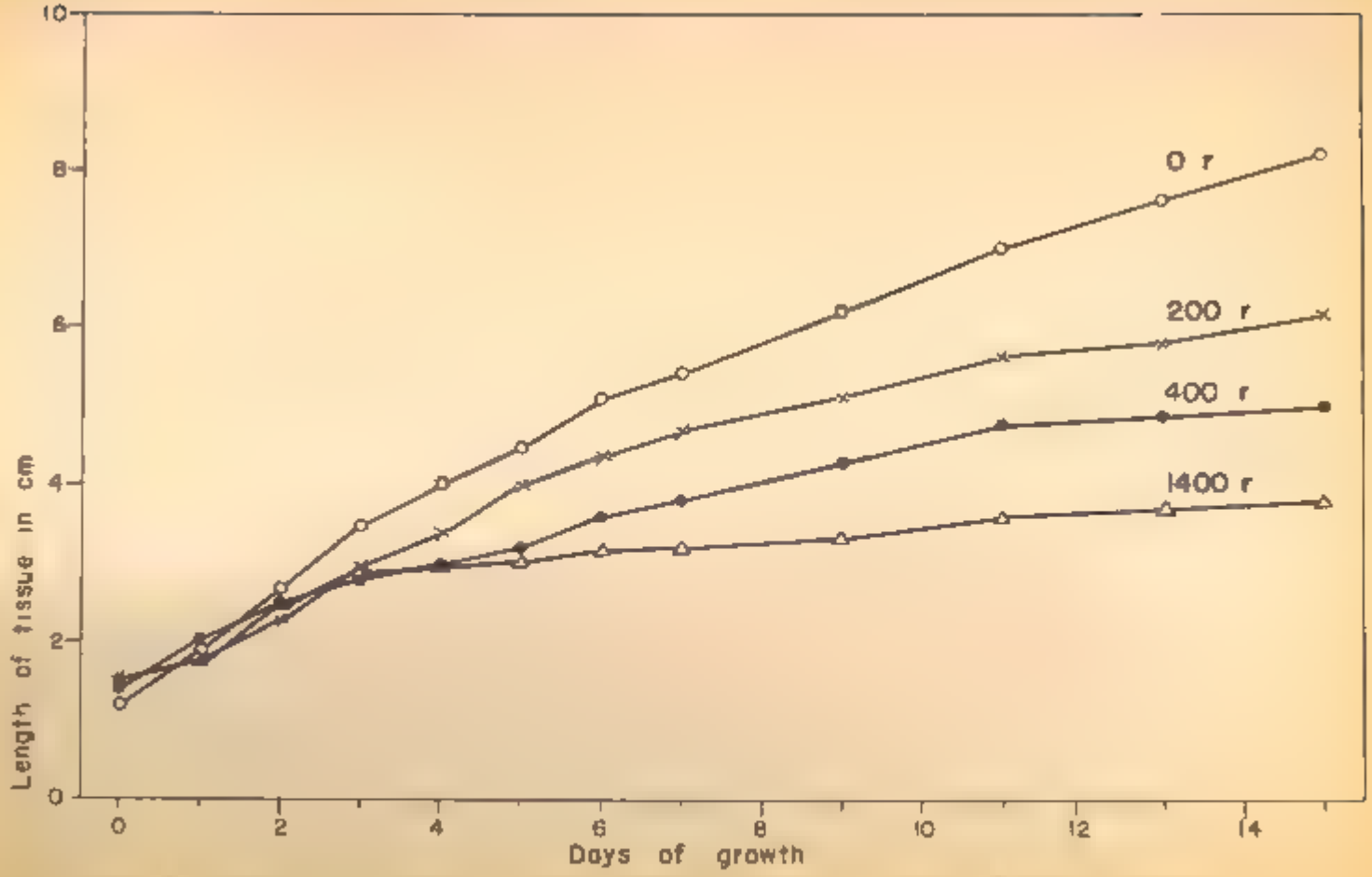

Fig 1 Lengths of irradiated tissue cultures grown in sterile nutrient containing 10^{-5} mg IAA per liter

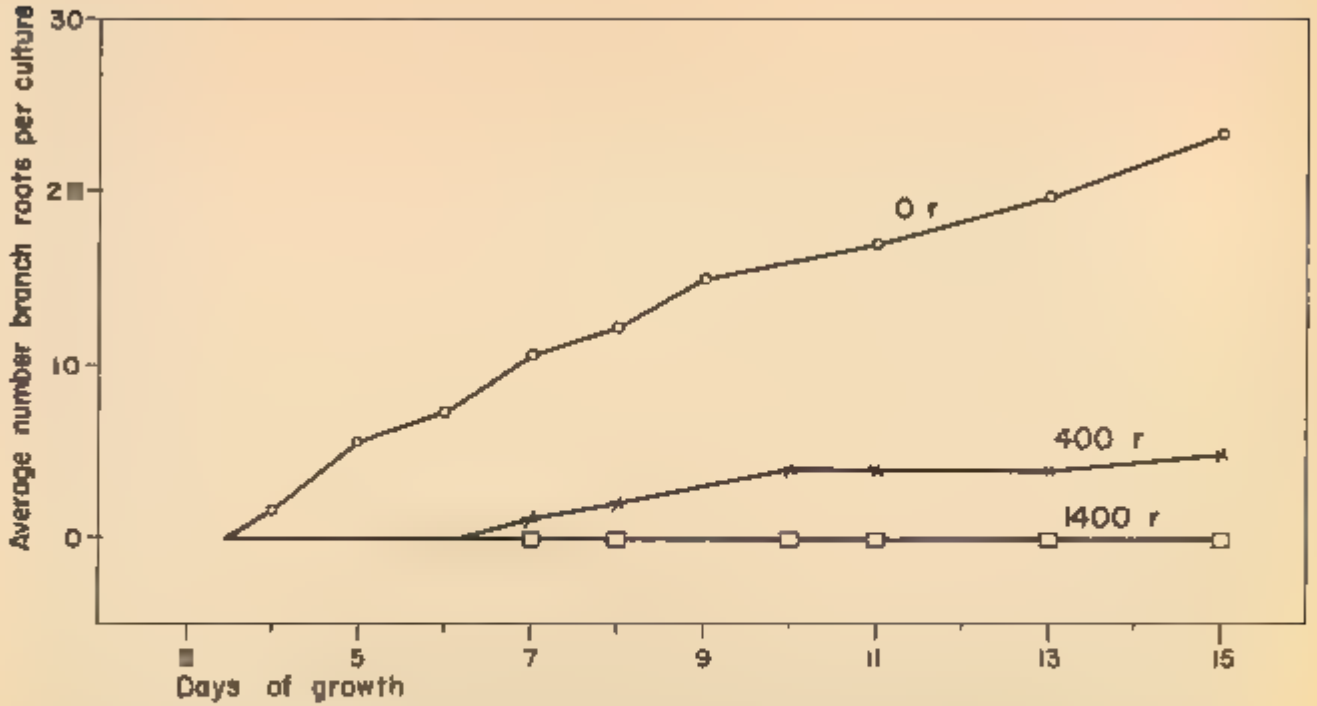

Fig. 2 — Average number of branch roots per culture irradiated excised tissues grown in sterile nutrient containing 10^{-5} mg IAA per liter.

[redacted]

Introduction and Experimental Procedure

The bioelectric potentials of different seeds and coleoptiles have been found to be depressed by X-rays (1, 2, 3). On the basis of previous investigations, it is to be expected that the potentials of excised corn roots would be lowered by radiation.

Excised corn roots are prepared for the experiment as outlined in Chapter IV of this report. Ten of the tissue cultures are given X-ray doses of 1400 r and ten cultures not subjected to radiation treatment are used as controls. The cultures are then grown in 125 ml Erlenmeyer flasks, containing 50 ml of White's nutrient medium with 10^{-5} mg per liter indoleacetic acid, for 24 hours at 30° C. At the end of this growth period the bioelectric potential, the length, and the diameter of each tissue culture are measured.

The results of the observations are given in Table 1. The mean lengths and diameters of the cultures are very nearly the same but the mean bioelectric potential of the irradiated group is about 20% lower than that of the control group. A test of the significance of the difference in the mean bioelectric potentials of the control and irradiated groups shows that the potentials are truly different. Thus, the decrease in the mean potential of the irradiated group can be attributed to the action of the radiation. The summary of the calculations for the significance test is given in Table 2.

TABLE 1

MEASUREMENTS OF THE BIOELECTRIC POTENTIALS OF CONTROL AND IRRADIATED EXCISED CORN ROOTS GROWN ON NUTRIENT MEDIA CONTAINING 10^{-5} MG IAA PER LITER

CONTROL			IRRADIATED (14,000r)		
Potential	Length	Diameter	Potential	Length	Diameter
40.5 mv	18 mm	1.0 mm	27.9 mv	18 mm	0.9 mm
45.0	19	0.8	27.0	15	0.5
36.9	20	1.0	29.7	14	0.8
32.4	12	0.5	25.2	19	1.0
37.8	17	1.0	31.5	19	0.9
38.7	20	0.8	27.0	20	0.8
39.6	18	0.9	27.0	15	1.0
27.9	16	1.0	36.0	14	0.5
39.6	13	0.9	27.9	18	0.9
28.8	17	1.0	32.4	18	0.8
S 367.2	170	8.9	291.6	170	8.1
M 36.7	17.0	0.89	29.2	17.0	0.81

[redacted]

SUMMARY OF THE CALCULATIONS TO DETERMINE THE SIGNIFICANCE OF THE DIFFERENCE IN THE BIOELECTRIC POTENTIALS OF CONTROL AND IRRADIATED TISSUE CULTURES

Item	Control	Irradiated
Mean Potential, $\bar{x}$	36.7	29.2
Variance of Mean, $V_{\bar{x}}$	2.916	1.066
Standard Deviation of Mean, $\sigma_{\bar{x}}$	1.71	1.03
$\bar{d}$ $(= \bar{x}_1 - \bar{x}_0)$	—	-7.5
$V_{\bar{x}_1} + V_{\bar{x}_0} = V_{\bar{d}}$	—	3.902
Standard Deviation of Difference, $\sigma_{\bar{d}}$	—	2.00
$t_{2.8} = \bar{d}/\sigma_{\bar{d}}$	—	3.75
Probability, $\bar{x}_1$ and $\bar{x}_0$ belong to same population	—	0.031

[redacted]

The standard deviations of the mean values of the potential for the control and X-rayed group of tissue cultures, indicate that it is possible to use only a few tissue cultures to determine the effect of radiation on the bioelectric potential. In accordance with other studies, this experiment indicates that the bioelectric potential of the tissues show the effect of radiation damage several days before the damaging effect on growth is observable. It is interesting to note that the cultures exposed to X-ray doses of 1400 r showed a 20% decrease in the bioelectric potential while their respiration is depressed nearly 70%. This indicates that the potential of the tissues is not too representative of the overall physiological activity.

[redacted]

The bioelectric potentials of the individual tissue cultures are measured by contact to the tissue through a salt water bridge. Ag-AgCl electrodes immersed in the 0.1N KCl solution are connected to a differential amplifier having a high input resistance. The potential of the culture is observed by noting the deflection of a meter connected to the output of the amplifier.

Some of the undesirable features of this system which could possibly lead to errors in the measurements of the potentials are:

1. The contact electrolyte may permeate over and through the tissue and may cause a short circuit of the specimen.

2. The necessity for high sensitivity of the D.C. amplifier works against the requirement of stability.

In order to avoid these objections an attempt has been made to develop a vibrating probe electrometer. The principle upon which the instrument functions is that when a difference of potential exists between the two plates of a condenser and one of the plates is vibrated at a known audio frequency, the change in capacitance between the vibrating plate and the fixed plate serves to induce an alternating potential on the moving plate (4). Thus, a small vibrating probe placed close to an organism, which is the source of a D.C. potential, would have an alternating potential induced upon it by virtue of the changing capacitance between the probe surface and the surface of the organism (5). The above disadvantages characteristic of the D.C. system may be avoided by use of this principle in the measurement of bioelectric potentials since no direct contact to the specimen is made except by the reference return electrode, which need not be a salt water contact, and A.C. amplifying circuits are used instead of the more troublesome D.C. [redacted].

Figure 1 is a block diagram showing the vibrating probe, the narrow band A.C. amplifier, and the other components necessary for operation of the probe. The output of the amplifier is displayed as a sine wave on the screen of a cathode ray oscilloscope. A Hewlett-Packard audio oscillator, type 200-B, drives a small two inch speaker at a frequency near 500 cycles per second. Firmly attached to the center of the speaker cone is a round plastic rod having a cross-sectional area of six square

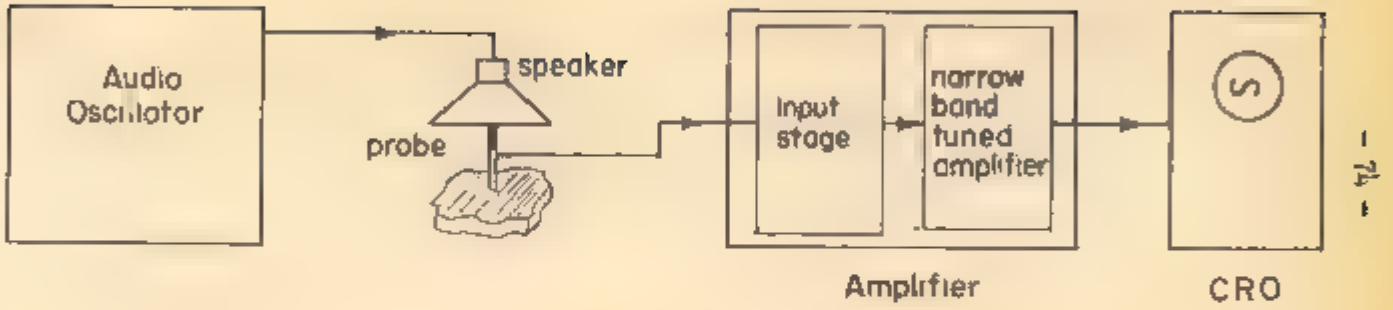

Figure 1. Block diagram of components of vibrating probe electrometer

millimeters. This rod is about ten centimeters long and vibrates in the vertical plane at the input audio frequency. The lower flat surface of the rod is coated with a smooth layer of conducting silver paint which is connected by a fine wire to the signal test lead leading to the input stage of the amplifier. The probe is placed as close to the specimen as is feasible.

Figure 2 is a schematic diagram of the amplifier. The input stage is completely shielded and contains an acorn tube, type 954, used as a cathode follower. A precision resistor of 10^8 ohms is used in the input grid circuit. A part of the output of the first tube is fed into the cathode follower stage, tube type 6AC7, whose output is used to drive the shielding of the input stage. Since the voltage gain of each of the two cathode follower stages is slightly less than unity, the shielding of the input stage is driven at a potential slightly less than that of the input signal (6). When the shielding of the input stage is floated at a potential near that of the signal, there is no significant leaking of the signal off to ground and the input capacitance appears to the signal source to be greatly reduced. Moreover, looking into the amplifier, the input resistance appears much larger because of the low signal current flowing in the input circuit.

The amplifier following the input circuit employs an LC type bridged T network in the feedback circuit and is a modification of one developed by Turner (7). This audio frequency amplifier provides a voltage gain of the input signal of about 2500 and it does not pass a signal of any frequency appreciably different from that for which it is

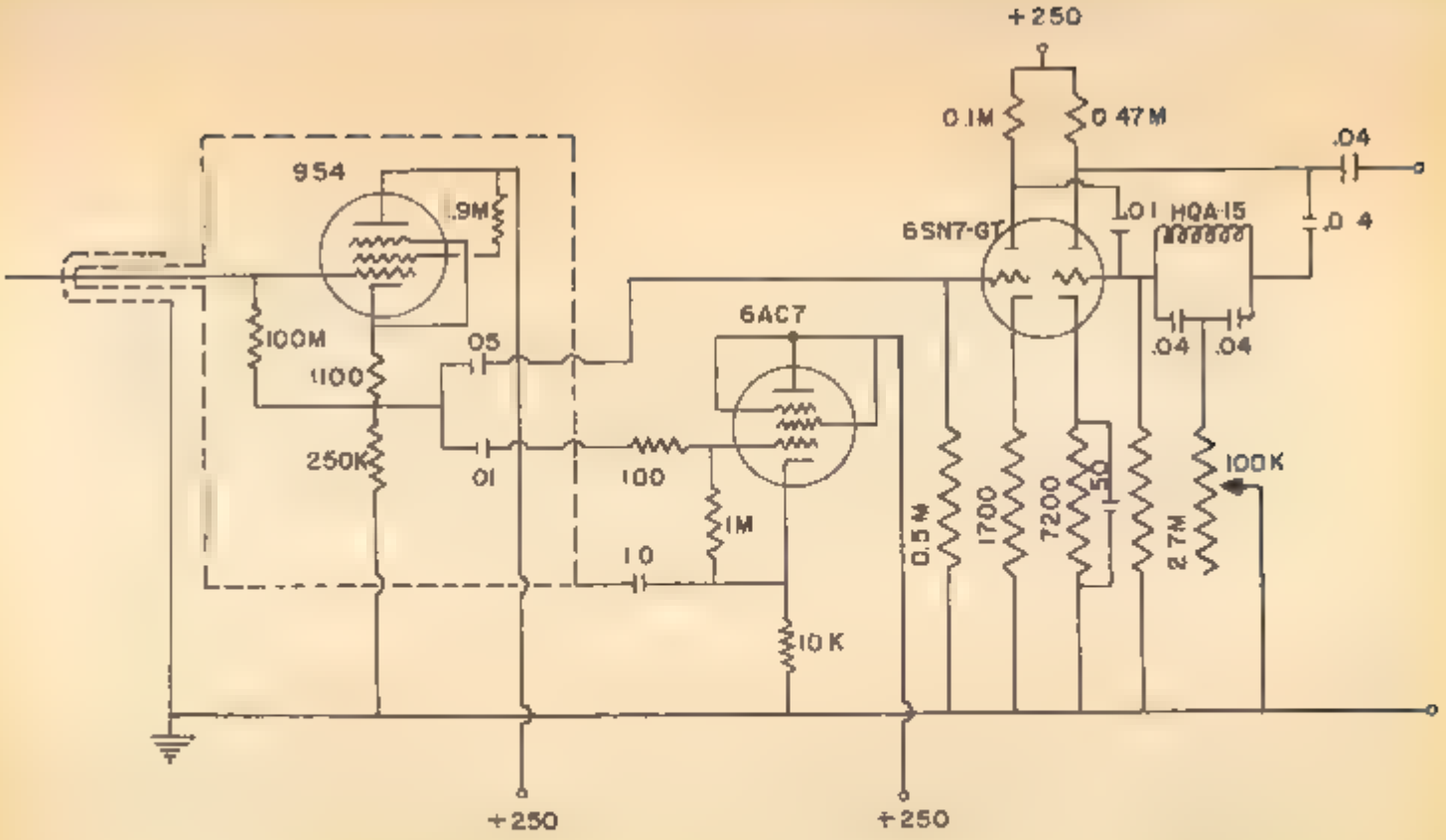

Fig 2 — The vibrating probe amplifier showing the driven input and narrow band tuned stages

tuned. Thus, spurious signals are prevented from reaching the final measuring instrument.

The sharpness of tuning of the amplifier is shown by the frequency response curve of Figure 3 which is obtained for one millivolt input to the grid of the first 6SH7 stage. The gain of the amplifier is down 30 db at 10 cycles off resonance. Sharpness of the amplifier tuning and voltage gain are indicated by the following response measurements: at 492 cycles the output is 2.55 volts for an input of 1 millivolt; at 491 cycles, 1.23 volts; and at 490 cycles, 0.66 volts. At 0.707 of the maximum resonant output, or 3 db down, the bandwidth is only two cycles.

Preliminary tests show that the amplifier is perfectly satisfactory for the detection of an A.C. signal having a potential much less than 1 mv; in fact, its noise level is about 30 microvolts. When the probe is not vibrating, a source of A.C. potential of 1 mv, placed about 1 mm distant from the end of the probe, is easily detected. However, on vibrating the probe a source of D.C. potential of 500 mv, placed as close to the probe surface as possible without contact, cannot be detected.

Previous circuits gave more promise in the measurement of the potentials. However, the above circuit is still in the developmental stage. One of the difficulties is believed to be the limited amplitude of vibration of the probe with the present equipment. The principle of operation is sound and it appears possible that on further development a working system can be evolved.

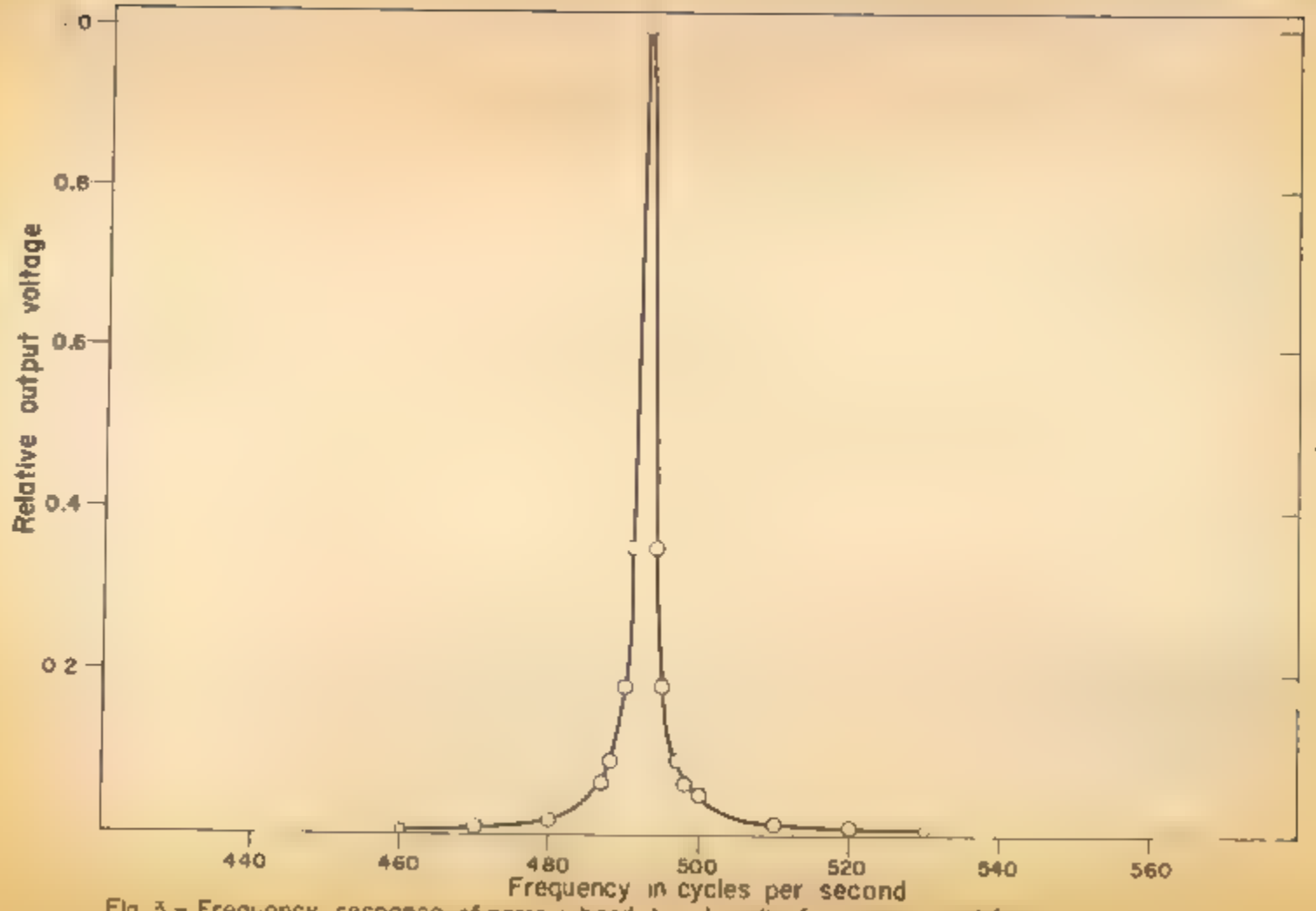

Fig 3 – Frequency response of narrow band tuned audio frequency amplifier

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SUMMARY AND CONCLUSIONS

From this work, concerned with various biophysical aspects of plant tissue cultures, the following may be concluded:

1. The primary growth of excised corn roots in sterile nutrient media is inhibited by indoleacetic acid concentrations of 10^{-1} and 10^{-2} mg per liter nutrient; it is enhanced by IAA concentrations of 10^{-5} and 10^{-6} mg per liter; it is unaffected by concentrations of 10^{-3} and 10^{-4} mg per liter.
2. The length and primary growth rate of excised corn roots increases regularly with decreasing auxin concentrations. It is possible that IAA helps to establish a constant growth rate for the cultures and, hence, may assist in the regulation of the rate of formation of new cells and of their elongation.
3. Indoleacetic acid plays an inverse role in the primary growth-lateral root formation relation of excised corn roots.
4. Indoleacetic acid in the concentrations of 10^{-6} to 10^{-1} mg per liter nutrient does not affect the respiration rate of excised corn roots grown in sterile nutrient solution.
5. The action of auxin and radiation on the processes of growth and respiration of excised corn roots is different.
6. The respiration rate of excised corn roots grown in sterile nutrient media is significantly lowered by X-radiation.

7. The respiration rate of the cultures most likely decreases exponentially with increasing X-ray dose.
8. The effects of radiation on the growth and respiration of excised corn roots are not due solely to the reduced activity of the auxin enzymatic system.
9. The primary growth of tissue cultures is inhibited by X-radiation.
10. Lateral root formation is strongly inhibited by radiation.
11. The bioelectric potential of excised corn roots is lowered by radiation. The potential is not indicative of the overall physiological activity of the cultures.

BIOGRAPHICAL SKETCH

Mark Wallen Jones was born in Wheeling, West Virginia, on December 20, 1916. He was graduated in June, 1938, from West Virginia University with a Bachelor of Arts degree. For eight years he was a staff member of the Department of Terrestrial Magnetism, Carnegie Institution of Washington. In the summer of 1947 he enrolled in the graduate school of the University of Florida. He was granted the Master of Science degree by the Department of Physics in June, 1948. For the next two years he was associated with the Geophysical Institute, located at College, Alaska, in the capacity of Fellow of the Institute. In September, 1950, he returned to the University of Florida to pursue studies in Physics and Biophysics leading to the degree of Doctor of Philosophy.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

January 31, 1953

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Dean, College of Arts and Sciences

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